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Effects of 7-OH-DPAT and NBQX on Reward and Locomotion in Rats

by

Anna-Maria Biondo

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Psychiatry

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ABSTRACT

Interactions between dopamine and glutamate in the nucleus accumbens (NAS) may play a role in schizophrenia, motivation and reward-related behaviours. The purpose of this study was to examine the systemic and central interaction between the dopamine D_2/D_3 receptor agonist 7-OH-DPAT and the glutamate AMPA receptor antagonist NBQX using measurements of place conditioning and locomotor activity in male rats. 7-OH-DPAT (0.03-5.0 mg/kg, s.c.) failed to induce place conditioning when administered immediately prior to conditioning; when administered 15 minutes prior to conditioning only the highest dose (5.0 mg/kg, s.c.) induced place preference. Microinjection of NBQX (0.5 μg) into the NAS shell blocked the acquisition of 7-OH-DPAT-induced place preference. In contrast, intra-NAS shell administration of 7-OH-DPAT (5.0 μg) or NBQX (0.5 μg), alone or in combination, failed to induce place conditioning. In addition, low doses of 7-OH-DPAT (0.03 and 0.06 mg/kg, s.c.) induced hypolocomotion whereas higher doses (1.67 and 5.0 mg/kg, s.c.) induced hyperlocomotion. These results suggest that stimulation of dopamine D_2 receptors by higher doses of 7-OH-DPAT may induce hyperlocomotion and place preference while activation of dopamine D_3 autoreceptors or inhibitory postsynaptic receptors by low doses of 7-OH-DPAT may contribute to hypolocomotion and lack of place conditioning. The blockade of 7-OH-DPAT-induced place preference by NBQX may be due to a synergistic relationship between dopamine D_2 receptors and glutamate AMPA receptors in the NAS shell.

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DEDICATION

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ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
7-OH-DPAT	7-hydroxy-N,N-di-n-propyl-2-amino-tetralin
ACPD	1-amino-cyclopentane-1,3-dicarboxylic acid
AMPA	2-amino-3-(3-hydroxy-5-methyl-4-isoxazolo)-propionic acid
AMPH	(+)-amphetamine sulfate
ANOVA	analysis of variance
AP	anterior-posterior
AP4	2-amino-4-phosphonobutyrate
CCK	cholecystokinin
CNQX	6-cyano-7-nitro-1,2,3,4-tetrahydro-quinoxaline-2,3-dione
CPA	conditioned place aversion
CPP	conditioned place preference
CSF	cerebrospinal fluid
DNQX	6,7-dinitroquinoxaline-2,3-dione
DV	dorsal-ventral
Ibo	ibotenate
i.p.	intraperitoneal
GABA	γ-aminobutyric acid
Glu	glutamate
L-DOPA	L-3-4-dihydroxyphenylalanine
mg	milligram(s)
MK-801	(±)-5-methyl-10,11-dihydro-5H-dibenzo[a,b] cyclohepten-5,10-imine, dizocilpine
mL	millilitre(s)
NAS	nucleus accumbens septi
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide
NMDA	N-methyl-D-aspartic acid
PCP	phencyclidine
PET	positron emission tomography

PFC	prefrontal cortex
QA	quisqualate
RNA	ribonucleic acid
s.c.	subcutaneous
SOP	serine-O-phosphate
VTA	ventral tegmental area
µL	microlitre(s)

1. GENERAL INTRODUCTION

Despite a century of probing the psychological and biological mechanisms of schizophrenia, the etiology of the disorder still remains a mystery. However, recent findings have implicated abnormal interactions between the monoamine neurotransmitter dopamine and the excitatory amino acid glutamate in the nucleus accumbens (NAS) as a potential underlying mechanism (Grace 1991; Csernansky and Bardgett 1998). The NAS receives dopaminergic input from the ventral tegmental area (VTA) and glutamatergic input from the prefrontal cortex (PFC) (Ikemoto and Panksepp 1999). Alterations in these projections may contribute to the symptoms of schizophrenia (Csernansky and Bardgett 1998). In order to gain a full and accurate understanding of the dopamine-glutamate hypothesis of schizophrenia, a brief review of dopamine and glutamate receptor subtypes, receptor distribution, and pharmacology as well as an examination of the structure and function of the NAS must be undertaken.

1.1. Background Information

1.1.1. Dopamine

Dopamine is a catecholamine neurotransmitter in the central nervous system. Dopamine receptors are of considerable interest as they are a primary target of drugs involved in the treatment of schizophrenia and Parkinson's disease (Kinon and Lieberman 1996), and they are linked to neuronal processes of learning, reward (Willner et al. 1991; Schultz 1997; Spanagel and Weiss 1999) and the control of movement (Zhang et al. 1997). Dopamine is synthesized from the aromatic amino acid *para*-tyrosine through a two-step process: first, *para*-tyrosine is converted into L-3-4-

dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase. The second step involves the decarboxylation of L-DOPA, catalyzed by the enzyme aromatic L-amino acid decarboxylase, to produce dopamine. Neuronal projections originating in areas of the brain that synthesize the neurotransmitter dopamine give rise to four axonal pathways: (1) tuberoinfundibular, (2) nigro-striatal, (3) mesocortical and (4) mesolimbic. Central dopaminergic pathways are summarized in Table 1.

Five distinct dopamine receptors have been identified and subdivided into two subfamilies, D_1-like and D_2-like. The dopamine D_1-like receptors are composed of D_1 and D_5 receptor subtypes, while the dopamine D_2-like receptors include D_2, D_3 and D_4 receptor subtypes. Dopamine D_1-like receptors stimulate adenylate cyclase activity when activated, whereas dopamine D_2-like receptors inhibit adenylate cyclase activity (Emilien et al. 1999). Dopamine receptors have a widespread distribution throughout the brain. Dopamine D_1 receptors have been identified in the NAS, olfactory tubercle, cerebral cortex, amygdala, islands of Calleja and the subthalamic nucleus. Dopamine D_5 receptors have a more restricted expression; they have been detected in the hippocampus, lateral mammillary nucleus and parafascicular nucleus of the thalamus (Jackson and Westlind-Danielsson 1994). Dopamine D_2 receptors are predominantly expressed in the caudate-putamen, olfactory tubercle, NAS and, to a lesser extent, in the substantia nigra pars compacta and VTA. The majority of dopamine D_3 receptors have been identified in the islands of Calleja, NAS and olfactory tubercle while moderate levels have been detected in the cerebral cortex, VTA, thalamic nuclei, ventral pallidum, superior colliculus and inferior olive (Levant 1997). Dopamine D_4 receptors have been

Table 1. Characteristics of central dopaminergic pathways

Pathway	Site(s) of Origin	Site(s) of Innervation	Function
Tuberoinfundibular	Periventricular and arcuate nuclei of the hypothalamus	Medial eminence of the hypothalamus	Inhibits the release of prolactin
Nigro-striatal	Substantia nigra pars compact	Dorsal striatum	Involved in the control of movement and its degeneration may cause Parkinson's disease
Mesocortical	Ventral tegmental area	Frontal cortex	Involved in some aspects of learning and memory
Mesolimbic	Ventral tegmental area	Nucleus accumbens, olfactory tubercle and parts of the limbic system	Influences motivated behaviour

(Adapted from Lindvall and Bjorklund 1978)

noted in the frontal cortex, amygdala, olfactory bulb, hippocampus, hypothalamus and mesenchephalon (Jackson and Westlind-Danielsson 1994).

1.1.2. Glutamate

L-Glutamic acid is an excitatory neurotransmitter in the central nervous system. Four subtypes of glutamate receptors have been identified, the G-protein coupled metabotropic receptors and three ionotropic subtypes namely N-methy-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA). The metabotropic glutamate receptors are structurally composed of seven transmembrane regions and are involved in the modulation of second messenger systems (Pin and Duvoisin 1995; Trist 2000). Cloning studies have revealed eight metabotropic glutamate receptor subtypes (mGluR1-mGluR8) that have been classified into three subgroups (group I-III) based on similarity in sequence homology, signal transduction mechanisms, and agonist selectivity (Pin and Bockaert 1995; Pin and Duvoisin 1995). Group I (mGluR1 and mGluR5) receptors activate phospholipase C whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) receptors inhibit adenylyl cyclase activity (Pin and Bockaert 1995). In addition, group I receptors appear to be located on postsynaptic membranes causing increased cell excitability while group II and III receptors are presynaptic modulators that inhibit glutamate release (Conn and Pin 1997; Bressan and Pilowsky 2000). Metabotropic glutamate receptors are summarized in Table 2.

The ionotropic glutamate receptors are made up of four or five subunits that form ligand-gated channels that open to allow the passage of small cations, such as Na^+ and

Table 2. Characteristics of metabotropic glutamate receptors

Subgroup	Receptor Subtype	Transduction mechanism	Agonist selectivity
I	mGluR1 mGluR5	Stimulates phospholipase C	QA>Glu≥Ibo>1S,3R-ACPD>AP4
II	mGluR2 mGluR3	Inhibits adenylyl cyclase	Glu≥1S,3R-ACPD>Ibo>QA>AP4
III	mGluR4 mGluR6 mGluR7 mGluR8	Inhibits adenylyl cyclase	AP4>SOP>Glu>Ibo>QA>1S,3R-ACPD

QA, Quisqualate; Glu, Glutamate; Ibo, Ibotenate; 1S,3R-ACPD, 1-amino-cyclopentane-1,3-dicarboxylic acid; AP4, 2-amino-4-phosphonobutyrate; SOP, Serine-O-phosphate (Adapted from Cotman et al. 1995; Pin and Bockaert 1995; Pin and Duvoisin 1995)

Ca^{2+} into the cell. AMPA receptor channels are formed by reconstituting one or any two of four subunits (gluR1-4) while the kainate subclass of receptors includes gluR5-7 and KA1-2 subunits (Cotman et al. 1995; Bressan and Pilowsky 2000). NMDA receptors are heteromeric protein ion channels composed of varying assemblies of 8 subunits, including NR1, NR2(A-D) and NR3(A-B) (Cotman et al. 1995; Gibb 2001; Goff and Wine 1997). AMPA and kainate receptors are suggested to mediate fast excitatory synaptic transmission while NMDA receptors mediate slow synaptic transmission. Ionotropic glutamate receptors are summarized in Table 3.

Glutamate receptors occur throughout the mammalian brain. AMPA receptor density is the highest of the three ionotropic receptors (Tamminga 1999); these receptors are predominantly distributed in the cortex, NAS, cerebellum, and temporal lobe structures such as the hippocampus, amygdala and, to a lesser extent, the thalamus (Cotman et al. 1987; Bettler and Mulle 1995; Cotman et al. 1995). Kainate receptors are enriched in the hippocampus, striatum, cortex, and areas of the thalamus (Cotman et al. 1987; Bettler and Mulle 1995; Cotman et al. 1995). NMDA receptors usually coexist with AMPA or kainate receptors as NMDA channels are blocked by Mg^{2+} at resting potential and require sufficient concurrent depolarization of the postsynaptic neuronal membrane to remove the Mg^{2+} and allow the NMDA channel to contribute to the electrical response of the cell. Typically, the level of simultaneous depolarization depends on AMPA or kainate receptor activation. The glycine site of the NMDA receptor complex must also be occupied by a co-agonist in order for glutamate to activate the cation channel (Dingledine et al. 1999). NMDA receptors appear to be concentrated

Table 3. Characteristics of ionotropic glutamate receptors

Receptor Subtype	Subunits	Cation Selectivity	Agonists	Antagonists
AMPA	GluR1-GluR4	Na^+, K^+	AMPA	CNQX DNQX NBQX
Kainate	GluR5-GluR7 KA1,2	Na^+, K^+	Kainate Domoic acid	CNQX DNQX
NMDA	NR1 NR2(A-D) NR3(A-B)	Na^+, K^+, Ca^{2+}	NMDA Glycine	D-AP5 PCP MK-801

(Adapted from Bettler and Mulle 1995; Cotman et al. 1995; Dingledine et al. 1999; Gibb 2001)

in limbic structures. The metabotropic glutamate receptors are widely distributed throughout the brain, but the subtypes are differentially distributed. Group I receptors have been found in abundance in hippocampus, amygdala, olfactory bulb, thalamus, cortex, striatum and NAS (Abe et al. 1992; Martin et al. 1992). Group II receptors have been identified in the cerebral cortex, thalamic reticular nucleus, caudate-putamen, supraoptic nucleus and the cortico-striatal glutamate projection (Ohishi et al. 1993; Cotman et al. 1995) whereas group III receptors appear to be localized in the lateral septum, thalamus, pontine nucleus and entorhinal cortex (Kristensen et al. 1993; Tanabe et al. 1993; Cotman et al. 1995).

1.1.3. The Nucleus Accumbens (NAS)

1.1.3.1. Heterogeneity of the NAS

The nucleus accumbens (NAS) is a bilateral structure located in the striatum of the basal forebrain. It has been associated with several functional roles such as the regulation of motor output, emotional and motivational processes and reward. An abundance of evidence has demonstrated that the NAS is a heterogeneous structure that can be divided into two primary subregions, the core and the shell (Heimer et al. 1991; Meredith et al. 1992; Pennartz et al. 1992; Zahm 1999; Heidbreder and Baumann 2001). These areas differ in afferent and efferent connections (Chronister et al. 1980; Heimer et al. 1991; see review by Brog et al. 1993; Groenewegen et al. 1999; Otake and Nakamura 2000), function (Heimer et al. 1991; Zahm and Brog 1992), and neurochemistry. For example, the core and shell receive different combinations of afferent projections from

the PFC, thalamus, amygdala, hippocampus and the VTA (Groenewegen et al. 1999). The core has extensive efferent connections with the dorsalateral ventral pallidum, subthalamic nucleus and substantia nigra whereas the shell has efferent pathways to the lateral hypothalamus and extended amygdala (Heimer et al. 1991; Ikemoto and Panksepp 1999). Based on these outputs, the core has been associated with motor system activity while the shell has been linked to limbic functions such as motivated behaviours (Heimer et al. 1991; Zahm and Brog 1992); these functions have been proposed to be mediated by mesolimbic dopaminergic projections (Canales and Iversen 2000). Higher concentrations of dopamine D_2 receptors are present in the core whereas greater abundance of dopamine D_1 and dopamine D_3 receptors are present in the shell (Bardo and Hammer 1991; Le Moine and Bloch 1996). Dopamine release in the shell and core has been found to respond differently to the administration of dopaminergic drugs. Microdialysis and voltammetry studies have shown that numerous drugs of abuse, such as amphetamine, nicotine, cocaine and morphine, preferentially increase extracellular dopamine in the shell as compared to the core (Pontieri et al. 1995; Pontieri et al. 1996; Nisell et al. 1997). Marcus and associates (1996; 2000) have indicated that systemic administration of haloperidol, a typical antipsychotic drug (dopamine D_2 receptor antagonist), increases core dopamine utilization whereas atypical antipsychotic drugs, such as clozapine, dramatically increase shell dopamine utilization. In addition, intravenous administration of the non-competitive NMDA antagonists, phencyclidine or dizocilpine (MK-801), elevated extracellular dopamine in the shell but not the core of the NAS whereas the competitive NMDA receptor antagonist CGP 39551 increased extracellular dopamine levels in the shell only at high doses (Marcus et al. 2001). Important differences exist for

glutamate interactions with dopamine in the NAS core and shell as contrasting dopamine-dependent (Moghaddam and Bolinao 1994; Svensson et al. 1994) and dopamine-independent (Balfour et al. 1996; Druhan et al. 1996) effects of glutamate receptor-related compounds on neurochemistry and behaviour have been reported. However, a recent paper by Jackson and associates (2001) suggested that physiologically relevant electrical stimulation of PFC neurons may inhibit the release of dopamine within the NAS, depending on the frequency of stimulation. That is, the PFC may exert inhibitory effects on dopamine release in the NAS; this finding appears to contradict neurochemical results discussed above. In turn, descending glutamate projections from the PFC to the NAS may have the potential to exert inhibitory control over the release of dopamine rather than facilitating its release. Taken together, these findings indicate that the relationship between glutamate and dopamine within the NAS needs further clarification.

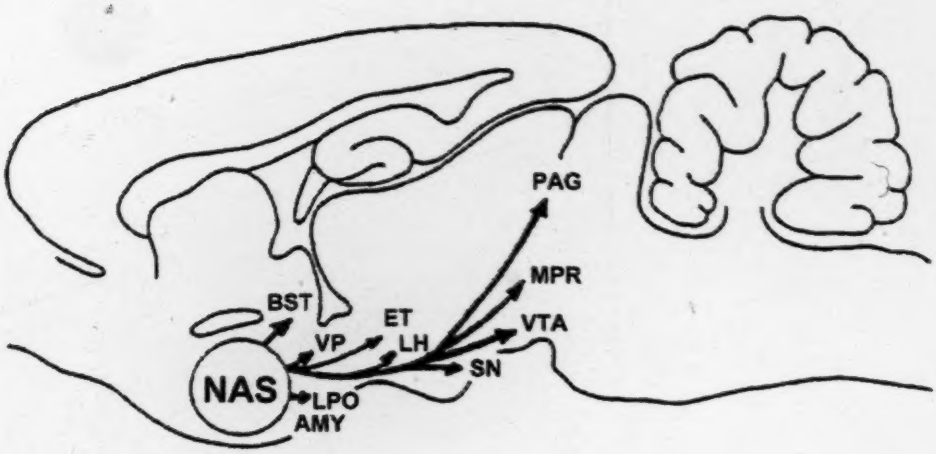
1.1.3.2. Neuronal Pathways in the NAS

Due to its neurochemical circuitry, the NAS has been conceptualized as a limbic-motor interface involved in the integration and processing of information related to motivation, emotion and motor responses (Mogenson et al. 1980; Meredith and Totterdell 1999). The NAS sends major efferent projections to a multitude of areas in the mammalian brain such as the VTA, ventral pallidum, preoptic area, lateral hypothalamus, entopeduncular nucleus and mesopontine reticular nuclei. Efferent projections are represented in Figure 1.1. In turn, the NAS receives afferent projections from a variety of brain regions; major inputs arise from the VTA, prefrontal cortex, basolateral amygdala,

Figure 1.1. Major efferent pathways of the NAS in the rat brain (sagittal representation)

AMY, amygdala; BST, bed nucleus of stria terminalis; ET, entopeduncular nucleus; LH, lateral hypothalamus; LPO, lateral preoptic area; MPR, mesopontine reticular nuclei; NAS, nucleus accumbens; PAG, periaquaductal grey; SN, substantia nigra; VP, ventral pallidum; VTA, ventral tegmental area

(Adapted from Ikemoto and Panksepp 1999)



paraventricular nucleus and parvoventricular nucleus of the thalamus, hippocampus and, mesopontine reticular nuclei (Ikemoto and Panksepp 1999). Afferent projections are represented in Figure 1.2. More specifically, neurochemical evidence has revealed that the NAS receives major descending "corticolimbic" glutamatergic input from the PFC and ascending "mesolimbic" dopaminergic input from the VTA. Dopaminergic and glutamatergic terminals in the NAS are believed to synapse on postsynaptic GABAergic medium spiny interneurons. That is, dopamine and glutamate terminals are in close apposition and synapse on GABAergic interneurons, thus forming a "synaptic triad" arrangement (Goldman-Rakic 1992). Numerous studies have proposed a reciprocal relationship between dopamine and glutamate within the NAS (see review by Morari et al. 1998). A recent investigation into the neuropathology of schizophrenia has focused on the reciprocal interaction between dopamine and glutamate within the NAS (Csernansky and Bardgett 1998).

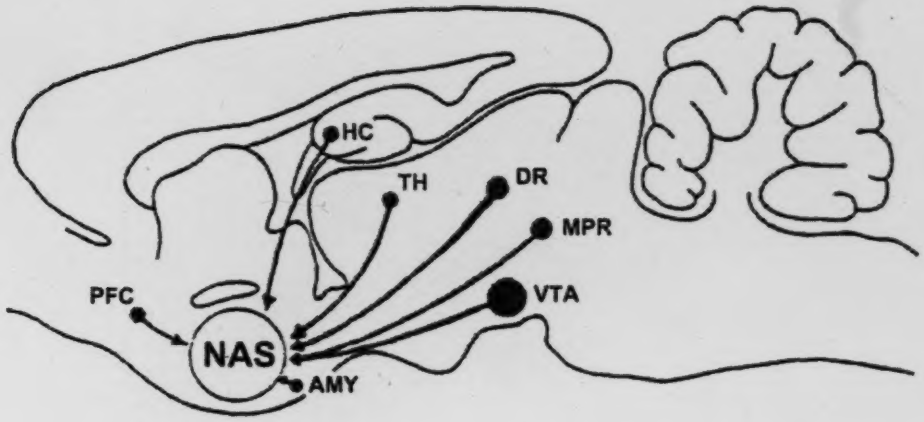
1.2. Dopamine and Glutamate Hypotheses of Schizophrenia

Schizophrenia is a puzzling and disabling psychiatric disorder that affects approximately 1% of the general adult population irrespective of culture, ethnicity or socioeconomic status. According to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV), the disorder is characterized by positive symptoms such as delusions, hallucinations, and thought disorders as well as negative symptoms such as poor social functioning, cognitive impairment, affective flattening or inappropriateness, and motor, volition, and behavioural disorders. Typically, the onset of illness occurs in late adolescence or early adulthood; males tend to show an earlier age of onset, early to

Figure 1.2. Major afferent pathways to the NAS in the rat brain (sagittal representation)

AMY, amygdala; DR, dorsal raphe; HC. hippocampus; MPR, mesopontine reticular nuclei; NAS, nucleus accumbens; PFC, prefrontal cortex; TH, thalamus; VTA, ventral tegmental area

(Adapted from Ikemoto and Panksepp 1999)



mid twenties, than their female counterparts. Marked gender differences have been reported in the risk and clinical expression of the disorder. For instance, males report experiencing more negative symptoms, specifically greater cognitive deficits and behavioural impairments (Goldstein and Tsuang 1990; Gorwood et al. 1995; Leung and Chue 2000), and show poorer long-term outcome whereas females are more likely to have an affected family member, experience more positive and affective symptoms and respond better to drug therapy (Goldstein 1997; Leung and Chue 2000). Up to 50% of schizophrenic patients attempt suicide, with 15% ultimately succeeding (Pearlson 2000; Rowley et al. 2001). In addition, substance abuse is also common among those diagnosed with schizophrenia (Chambers et al. 2001).

Despite a century of research, the etiology of schizophrenia remains elusive. Structural imaging studies have proposed that enlarged ventricles (Daniel et al. 1991; van Horn & McManus 1992; Lawrie and Abukmeil 1998) and reduction in the volume of temporal lobe and in medial temporal structures, consisting of the hippocampus, parahippocampal gyrus and amygdala, (Lawrie and Abukmeil 1998; Nelson et al. 1998) are linked to the neuropathology of schizophrenia. However, the majority of research has focused on neurochemical irregularities involving dopamine, glutamate, 5-hydroxytryptamine and, to a lesser extent, GABA as contributing to the underlying mechanisms of schizophrenia. The neurochemical abnormalities associated with dopamine and glutamate are reviewed below.

1.2.1. Dopamine Hypothesis of Schizophrenia

The dopamine hypothesis has dominated and guided biological schizophrenia research for several decades. It centers on the idea that the symptoms of schizophrenia are due to dopaminergic hyperactivity, especially in the mesolimbic dopamine pathway, arising from increased availability of dopamine or elevated sensitivity to dopamine (Littrell and Schneiderhan 1996; Harrison 1999). The majority of support for this hypothesis is based on pharmacological evidence. Early research revealed that chronic use of the psychostimulant amphetamine, an indirect dopamine receptor agonist, can induce behaviours that mimic paranoid schizophrenia (Connell 1958; Angrist and Gershon 1970). Typical antipsychotic drugs, such as haloperidol and chlorpromazine, antagonize the dopamine system by blocking dopamine D_2 receptors (Creese et al. 1976; Seeman et al. 1976; Chiodo and Bunney 1983; Farde et al. 1988). Postmortem studies have found increased density of striatal dopamine D_2 receptors in medicated schizophrenic patients (Lee et al. 1978; Owen et al. 1978; Seeman 1987). An increased number of dopamine D_2 receptors in the brains of medicated schizophrenic patients may contribute to enhanced dopaminergic neurotransmission. More recent findings have suggested that the dopamine D_3 receptors also express a high affinity for antipsychotic drugs; these receptors also have a preferential distribution in mesolimbic structures, such as the NAS (Sokoloff et al. 1990; Kaichi et al. 2000; Schwartz et al. 2000).

In addition, functional imaging studies have provided support for the dopamine hypothesis (see review by Seeman and Kapur 2000). For instance, positron emission tomography (PET) studies have revealed increased dopamine turnover in drug-naïve schizophrenic patients as compared to age-matched controls (Hietala et al. 1994; Dao-

Castellana et al. 1997). In turn, medicated schizophrenic patients have shown greater dopamine release in the striatum as compared to age-matched controls, as indicated by displacement of radiolabeled dopamine D_2 receptor antagonists following an amphetamine challenge. The increase in striatal dopamine has been correlated with the induction or worsening of positive symptoms (Laruelle et al. 1996; Breier et al. 1997; Laruelle et al. 1997; Abi-Dargham, 1998; Breier et al. 1998). That is, dopaminergic hyperactivity in mesolimbic regions may be the underlying cause of positive symptoms such as hallucinations and delusions.

Even though the presence of increased dopamine concentrations has been demonstrated in the brains of schizophrenic patients, it is becoming clear that dopamine is not the only neurotransmitter involved in the neuropathology of the disorder. For example, numerous antipsychotic medications, such as risperidone and olanzapine, have been developed that apparently exert therapeutic action through blockade of other neurotransmitter receptors such as 5-HT receptors (Meltzer et al. 1989). Review of biochemical, pharmacological, and imaging studies suggest that dopamine overactivity may not sufficiently explain the cause of negative symptoms or the lack of effectiveness of most antipsychotics in treating these symptoms.

1.2.2. Glutamate Hypothesis of Schizophrenia

Close interaction between neurotransmitters in the brain has raised the question as to whether dopamine is the only neurotransmitter involved in the etiology of schizophrenia. Considerable interest has emerged as to the possible role of glutamate in schizophrenia (see reviews by Tamminga 1999; Bressan and Pilowsky 2000; Carfagno et

al. 2000; Lewis and Lieberman 2000; Meador-Woodruff and Healy 2000). Much of this interest has stemmed from the findings that (1) glutamate concentrations are reduced in the cerebrospinal fluid of schizophrenic patients as compared to control subjects (Kim et al. 1980; Bjerkenstedt et al. 1985) [however numerous studies have not been able to replicate these findings (Perry 1982; Korpi et al. 1987; Tsai et al. 1998)] and (2) NMDA receptor antagonists, such as phencyclidine (PCP), ketamine and MK-801, produce both positive and negative symptoms commonly seen in schizophrenia in both schizophrenic patients and normal controls (Allen and Young 1978; Javitt and Zukin 1991; Krystal et al. 1994; Kegeles et al. 2000; Lahti et al. 2001). In general, post-mortem radioligand binding studies have indicated an increased density of NMDA and kainate receptor subtypes in the frontal cortex (Nishikawa et al. 1983; Toru et al. 1988; Deakin et al. 1989; Kornhuber et al. 1989) and putamen (Kornhuber et al. 1989). The increase in glutamate receptors may be due to a compensatory up-regulation of glutamate receptors in response to hypoglutamateric function in schizophrenia (Carfagno et al. 2000). Post-mortem studies have also shown decreased kainate and AMPA receptors in the hippocampus of schizophrenic patients (Kerwin et al. 1988; Kerwin et al. 1990). In addition, decreased messenger RNA for non-NMDA receptor subtypes in the temporal cortex, amygdala and hippocampus of schizophrenic patients has been found (Harrison et al. 1991; Eastwood et al. 1995; Eastwood et al. 1996). These findings suggest decreased glutamatergic activity as a possible mechanism underlying the symptoms of schizophrenia.

A primary mechanism used to explain the involvement of glutamate in schizophrenia centers on its interaction with dopamine (Toru et al. 1994; Csernansky and

Bardgett 1998; Carlsson et al. 1999; Moore et al. 1999; Floresco et al. 2001; Kegeles et al. 2000). Throughout the brain, dopaminergic neurons synapse with glutamatergic neurons (Sulzer et al. 1998); dopamine neurons appear to be modulated by cortical glutamatergic neurons either directly or indirectly via GABAergic interneurons (Goldman-Rakic 1992; Kretschmer 1999; Wu et al. 2000). Studies using laboratory animals confirm a functional interaction between dopamine and glutamate. For instance, a microdialysis study conducted by Miller and Abercrombie (1996) found dramatic enhancement of dopamine release following the administration of MK-801, a non-competitive NMDA antagonist, to amphetamine-treated animals. More specifically, animal studies have suggested that the activity of dopaminergic neurons in the striatum is regulated by glutamatergic projections from the PFC (Imperato et al. 1990; Burns et al. 1994). A microdialysis study conducted by Imperato and associates (1990) revealed that perfusion of the NAS with quisqualate and kainate, selective glutamate agonists, increased the release of dopamine in this area. Human neuroimaging studies by Breier and colleagues (1998) and Smith and associates (1998) revealed a decrease in dopamine D_2 receptor availability due to increased synaptic dopamine concentrations in normal subjects undertaking a ketamine challenge. Expanding on these findings, Adler et al. (1999) found even greater dopamine release in schizophrenic patients treated with ketamine as compared to normal controls, while Kegeles and associates (2000) noted that administration of ketamine increased amphetamine-induced dopamine release in the striatum and brought about negative schizophrenia symptoms in healthy volunteers.

Numerous hypotheses have emerged to explain the relationship between dopamine and glutamate. Grace (1991) suggested that decreased glutamate input to the

NAS reduced the tonic release of dopamine by decreasing dopamine autoreceptor activation, subsequently resulting in upregulation of phasic dopamine receptors. Psychotic symptoms associated with schizophrenia are potentially due to decreased glutamatergic input from the hippocampus and other limbic structures to the NAS (Carlsson and Carlsson 1990; Floresco et al. 2001). In turn, Csernansky and Bardgett (1998) have suggested that schizophrenic symptoms may be due to elevated concentrations of mesolimbic dopamine resulting from damage to limbic-cortical glutamate neurons. The evidence that dopamine and glutamate abnormalities play a pivotal role in the neuropathology of schizophrenia has provided a basis for studying the interplay between dopaminergic and glutamatergic compounds using laboratory animals.

1.3. Animal Model of Schizophrenia

Schizophrenia has long been deemed a human psychiatric disorder with few comparable behaviours are evident in other animal species. Numerous animal models have been developed to induce behaviours believed to parallel the symptoms associated with schizophrenia; however, these models have yet to fully capture the myriad of symptoms experienced by schizophrenics (Lyon 1991). Reward-related tests have been used to model anhedonia, an aspect of schizophrenic symptomatology that presents in schizophrenic patients as an inability to experience the rewarding aspects of normally pleasurable stimuli associated with everyday activities, such as sexual relations. As discussed in Section 1.4.1., CPP is an experimental paradigm that makes use of the phenomenon of secondary conditioning (Carr et al. 1989; Phillips and Fibiger 1987; van der Kooy 1987; Bardo and Bevins 2000). It centres on the assumption that when a

neutral stimulus is paired with rewarding/aversive stimuli, the initially neutral stimulus gains the capacity to elicit the unconditioned behavioural effects initially associated with the rewarding/aversive stimuli and in turn brings about approach responses and maintenance of contact on subsequent exposures (Carr et al. 1989; Hoffman and Beninger 1989; Phillips and Fibiger 1987; Tzschentke 1998). For that reason, the CPP paradigm can be used to explore the neuropharmacological basis of reward processes in an attempt to explore systems relevant to anhedonia in schizophrenia.

The symptoms of schizophrenia maybe due to increased dopaminergic activity within the nucleus accumbens. This may be related to a decrease in glutamate neurotransmission. Increased dopamine neurotransmission may be linked to the induction of reward-related behaviours (as discussed earlier). Thus, investigation of the effects of dopamine receptor agonists and glutamate receptor antagonists on CPP may help to define the role of these neurotransmitters in secondary reinforcement, and possibly anhedonia. It is predicted that dopamine receptor agonists will elicit CPP as the rewarding properties of these drugs would become associated with the environmental stimuli. If an increase in dopaminergic function were caused by decreased glutamate receptor activity, it would be expected that CPP induced by a dopamine agonist would be potentiated by the administration of a glutamate receptor antagonist. It is based on these hypotheses that the experiments in this thesis are based.

1.4. Thesis Objectives

Hyperdopaminergic and hypoglutamatergic functions, particularly within the NAS, have been implicated in the neuropathology of schizophrenia. That is, abnormal interactions between PFC glutamate and mesolimbic dopamine in the NAS may contribute to the underlying etiology. Recent research has indicated that AMPA receptors and dopamine D_3 receptors may contribute to the positive and negative symptoms associated with the disorder. In order elucidate the role that each plays, an accurate understanding of the basic interplay between the two neurotransmitters and their receptors subtypes must be undertaken.

The objectives of this thesis were to investigate:

1. the effects of systemic 7-OH-DPAT, a dopamine D_2/D_3 receptor agonist, on locomotor activity
2. the effects of systemic 7-OH-DPAT on conditioned place preference (CPP).
3. the interaction between intra-NAS shell NBQX, a glutamate AMPA receptor antagonist, and systemic 7-OH-DPAT on CPP.
4. the interaction between NBQX and 7-OH-DPAT in the NAS shell on CPP.
5. the possible state-dependent effects of NBQX and 7-OH-DPAT in the NAS shell on CPP.

1.5. Methodology

1.5.1. Reward and Conditioned Place Preference

Neurochemical evidence has suggested an abnormal interaction between dopamine and glutamate within the NAS as a potential mechanism underlying the symptoms of schizophrenia. Based on this hypothesis, researchers have turned to studies using laboratory animals to gain a basic understanding of the interplay that occurs between these neurotransmitters and their receptor subtypes. One experimental protocol that has been used to examine neural circuits by employing specific agonists and antagonists to identify the involvement of particular neurotransmitters and receptor populations is conditioned place preference (CPP). CPP is an experimental paradigm used to measure the rewarding or aversive properties of drugs in laboratory animals (Carr et al. 1989; Phillips and Fibiger 1987; van der Kooy 1987; Bardo and Bevins 2000). It centers on the assumption that when a neutral stimulus is paired with rewarding/aversive stimuli, the initially neutral stimulus gains the capacity to elicit the unconditioned behavioural effects initially associated with the rewarding/aversive stimuli and in turn brings about approach responses and maintenance of contact on subsequent exposures (Carr et al. 1989; Hoffman and Beninger 1989; Phillips and Fibiger 1987; Tzschentke 1998). That is, the paradigm makes use of the phenomenon of secondary conditioning in which a neutral stimulus paired with a reward acquires the capacity to serve as a reward itself.

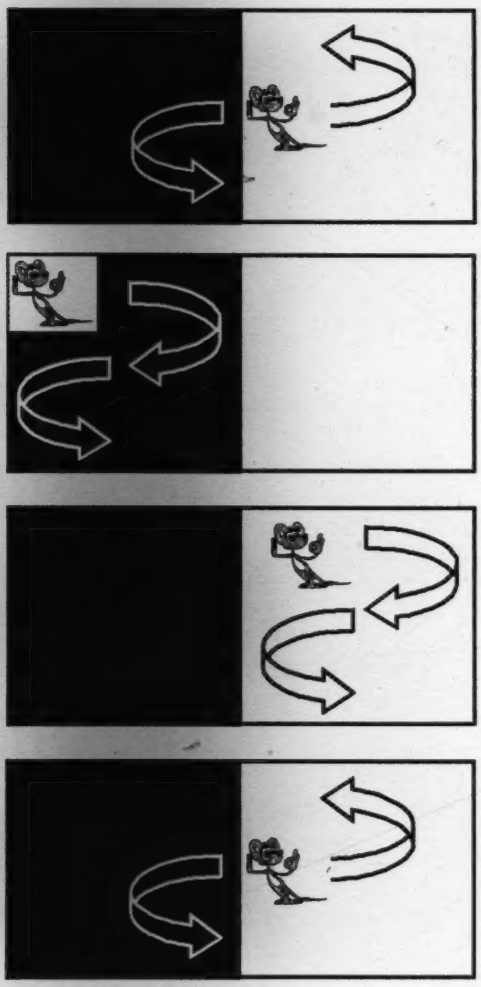
With the CPP method, animals are exposed to two distinct neutral environments; one environment is paired with the administration of drug while the other is paired with

the administration of vehicle. The primary incentive properties of the drug and non-drug treatments are repeatedly paired with a neutral set of environmental stimuli. Animals are later provided with the opportunity to enter both environments and the proportion of time spent in each is used as an index of reward (Swerdlow et al. 1989; Wise and Hoffman 1992; Tzschentke 1998; Bardo and Bevins 2000). Development of place preference (or aversion) is typically indicated by an increase (or decrease) in the amount of time spent in the drug-paired environment relative to time spent in the vehicle-paired compartment. According to Swerdlow and associates (1989) three distinct processes must occur in order for place preference or aversion to emerge: first, the drug must elicit a change in the internal affective state of the animal. The animal must then make an association between the affective change and distinct environmental cues, typically known as incentive learning. Finally, the animal must recall this association in the absence of the drug and use this memory to direct its behaviour. A simple version of the CPP procedure is summarized in Figure 1.3.

1.5.1.1. Involvement of Dopamine and Glutamate

Much interest has emerged as to the neurotransmitters and brain structures involved in reward processes; the CPP paradigm has served as a useful tool in analyzing the neural mechanisms of drug reward. The majority of research has implicated dopamine as an important neurotransmitter involved in mediating motivation and reward-related behaviours (Wise and Rompre, 1989; Tzschentke 1998). Drugs of abuse such as amphetamine (Spyraki et al. 1982; Biala and Langwiński 1996; Bardo et al. 1999) and cocaine (Biala and Langwiński 1996) that enhance dopamine transmission have

Figure 1.3. Simple version of CPP paradigm



Preconditioning

-animals exposed to entire apparatus for 15 minutes per day over three days in a drug-free state

Conditoning

-animals injected with drug on days 1,3,5,7 and restricted to one compartment

-animals injected with vehicle on days 2,4,6,8 and restricted to other compartment

Postconditioning

-animals exposed to the entire apparatus for 15 minutes per day over three days

Animals experience two distinct neutral environments, represented by black and white shading, paired with distinct unconditioned stimuli (drug on days 1,3,5,7 and vehicle on days 2,4,6,8). After conditioning animals are given the opportunity to enter either environment, and the amount of time spent in each environment is used as an index of the rewarding value of each unconditioned stimulus (drug or vehicle).

consistently been found to produce CPP. The dopamine agonists apomorphine and quinpirole have also been demonstrated to induce CPP (White et al. 1991; Funada and Shippenberg 1996) whereas the dopamine antagonists SCH23390 and SCH39166 have been found to produce conditioned place aversion (CPA) (Acquas and Di Chiara 1994; Cervo and Samanin 1995). It should be noted that these results are not consistently found across all dopaminergic agonists and antagonists. In addition, dopamine receptor antagonists such as haloperidol, pimozide and SCH23390 have been found to attenuate amphetamine and cocaine-induced CPP (Spyraki et al. 1982; Mithani et al. 1986; Hoffman and Beninger 1989; Baker et al. 1998). A recent study by Leri and Franklin (2000a) found that the benzodiazepine diazepam, which has been suggested to decrease dopaminergic turnover, prevents the acquisition and expression of amphetamine-induced CPP. Taken together, these findings support the assertion that dopamine receptor activation plays a central role in reward processes.

Building on the assumption that dopamine is a critical neurotransmitter in the development and mediation of reward-related behaviours, attempts have been made to identify the neuroanatomical substrate involved. The NAS has been implicated as an essential structure; more specifically, the "mesolimbic" dopaminergic pathway projecting from the VTA to the NAS has been suggested to play a major role. Numerous studies have indicated that bilateral microinjections of amphetamine, an indirect dopamine agonist, into the NAS reliably produced CPP in rats (Carr and White 1983, 1986; Josselyn and Beninger 1993; Schildein et al. 1998). As the NAS is considered a heterogeneous structure composed to two primary subareas, core and shell, Liao and colleagues (2000) investigated the effects of amphetamine infused into each region on the

acquisition of CPP. A significant place preference was observed with intra-NAS core infusions but not with shell injections. In addition, the authors demonstrated a marked CPP with cocaine when injected into the shell but not the core. These findings indicate that the rewarding effects of drugs may be dependent on differences in dopamine receptor distribution and efferent projections within the neural substrates of the NAS. That is, the mechanisms of action of particular drugs may be linked to receptor subtypes and dopaminergic neurotransmission within the core and shell. Leri and Franklin (2000b) have demonstrated that intra-NAS injections of diazepam block the expression of amphetamine-induced CPP. The lack of place preference may be due to interruption in the dopamine-dependent effects of amphetamine within the mesolimbic dopamine system. However, due to the extensive interplay between neurotransmitters in the NAS, it is unlikely that mesolimbic dopamine is the sole mediator of reward processes. Studies have suggested that regulation of reward within the NAS involves interaction between dopaminergic and glutamatergic neurotransmission.

There has been increased interest in the role of excitatory amino acids, specifically glutamate, in development and expression of CPP induced by psychostimulants and opiates. A recent investigation has reported that the development and expression of CPP are dependent on glutamatergic transmission and that NMDA and AMPA receptors play unique roles in the process (Mead and Stephens 1999). Studies by Tzschentke and Schmidt (1995, 1997) and Kim and associates (1996) have found that systemic administration of the non-competitive NMDA receptor antagonist MK-801 blocked morphine-induced CPP, while Suzuki et al. (2000) have revealed that ketamine, a non-competitive NMDA antagonist, suppressed CPP induced by morphine. In addition,

Cervo and Samanin (1995) and Kotlińska and Biala (2000) demonstrated systemic administration of MK-801 or memantine prevented the induction of cocaine-induced CPP, respectively. Intra-NAS administration of DNQX, an AMPA/kainate antagonist, inhibited the acquisition of amphetamine-induced (Layer et al. 1993) and cocaine-induced CPP (Kaddis et al. 1995). Based on these studies, the underlying mechanism necessary for psychostimulant- or opiate-induced CPP may be related to the interplay between dopamine and glutamate within the NAS.

1.5.2. Involvement of Dopamine and Glutamate in Locomotor Activity

Spontaneous locomotor activity has typically been used to gain insight into the depressant and stimulant effects of drugs. As with reward-related behaviour, the NAS is believed to play a major role in the control of exploratory motor responses. Several studies have implicated mesolimbic dopamine activity within the NAS as the primary neurochemical modulator of locomotion (Swanson et al. 1997; Canales and Iversen 2000; David and Abraini 2001). Administration of dopamine D_1 agonist SKF 82589 and dopamine D_2/D_3 agonist quinpirole into the NAS shell or core increased locomotor activity, with higher levels of activity following shell stimulation (Swanson et al. 1997). A study by Gong and associates (1999) found that intra-NAS infusions of the dopamine D_1 agonist SKF 38393 or the dopamine D_2 agonist quinpirole increased locomotor activity. Subsequent research has indicated functional interactions between dopamine and glutamate within the NAS as a potential modulator of locomotor activity. Burns and colleagues (1994) found that intra-NAS infusions of CNQX, an AMPA receptor antagonist, or AP5, an NMDA receptor antagonist, blocked amphetamine-induced

hyperactivity, while NMDA and AMPA agonists attenuated the amphetamine-induced hyperactivity. Microinjections of the mGlu receptor agonist 1S,3R-ACPD into the NAS elevated locomotor activity; in turn, co-injection of amphetamine and 1S,3R-ACPD potentiated the locomotor effect of amphetamine (Kim and Vezina 1998). Taken together, these findings suggest that a dynamic relationship exists between dopamine and glutamate systems as measured by observations of spontaneous locomotor activity.

2. GENERAL METHODS

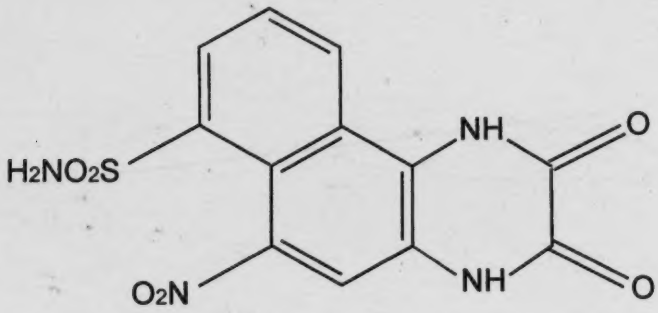
2.1. Subjects

Male Sprague-Dawley rats, weighing 200-250 g, were obtained from Health Sciences Laboratory Animal Services, University of Alberta. Animals were housed individually in Plexiglas cages in a temperature- (21±1°C) and humidity-controlled environment and maintained on a 12-hour light/dark cycle (lights on 0700-1900 hours). Food and water were freely available; standard animal chow (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc. Brentwood, MO, USA) was composed of 4.5% crude fat, 6.0% crude fiber, 23% crude protein, 8.0% ash and 2.5% added minerals. The care and use of animals in all experiments were carried out in accordance with guidelines set forth by the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council of Animal Care.

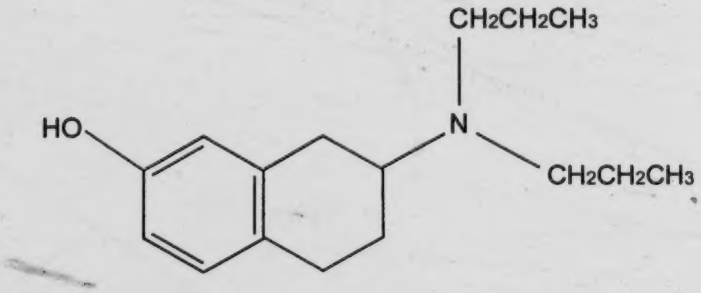
2.2. Drugs

The following drugs were used: (+)-amphetamine sulfate, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX) and 7-hydroxy-N,N-di-n-propyl-2-amino-tetralin hydrobromide (7-OH-DPAT-HBr). NBQX and 7-OH-DPAT-HBr were purchased from RBI (Natick, Mass., USA) whereas (+)-amphetamine sulfate was obtained from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). Chemical structures of the drugs are represented in Figures 2.1.

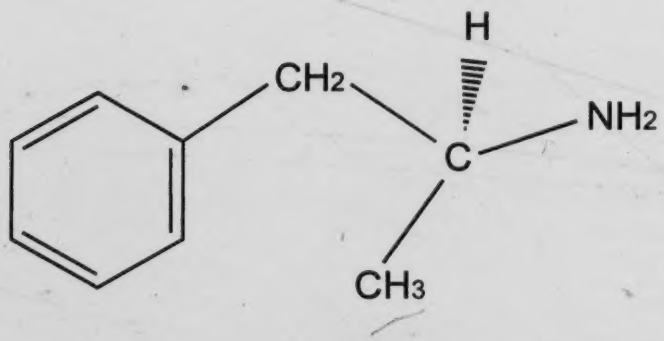
Figure 2.1. Structures of NBQX, 7-OH-DPAT and (+)-amphetamine



2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX)



7-hydroxy-N,N-di-n-propyl-2-amino-tetralin [(±)-7-OH-DPAT]



(+)-amphetamine

2.3. Locomotor Activity Measurements

Six computer-monitored photobeam activity boxes (I. Halvorsen System Design, Phoenix, AZ, USA) were used to measure spontaneous locomotor activity. Each box consisted of a Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) placed in a 12x12 infrared beam grid system 2.5 cm above the floor as well as 12 vertical sensors 12 cm above the floor. Three measurements were collected: total activity (total number of beam breaks, representing all locomotor behaviour), consecutive activity (successive breaking of the same beam, representing stereotyped behaviours), and vertical activity (number of upper photobeam breaks, representing rearing behaviour). Locomotor test sessions were conducted under red light illumination to induce higher basal activity in accord with prior studies from this laboratory.

2.4. Conditioned Place Preference

2.4.1. CPP Apparatus

The CPP apparatus (I. Halvorsen System Design, Phoenix, AZ, USA) consisted of a rectangular Plexiglas box divided into two equal-sized compartments (30 cm L x 30 cm W x 25 cm H). The compartments had distinct floor textures; one compartment had 1-cm square grate flooring while the other contained 14 horizontal bars arranged 1.25 cm apart. The compartments were separated by an opaque plastic partition that contained a 7.5-cm-long tunnel to allow access to both sides, with a removable door on each end.

2.4.2. CPP Procedure

The CPP procedure consisted of three phases: preconditioning (Phase 1), conditioning (Phase 2) and postconditioning (Phase 3). Testing was conducted under red light illumination in accordance with prior studies in this laboratory. The boxes were cleaned with an ammonia-based window cleaner (No Name, Club Pack, Glass Cleaner with Ammonia) that was diluted with six parts water to one part cleaner between animals. During Phase 1, animals were habituated to the CPP boxes for three days by allowing free access to both compartments for 15 minutes/day. The time spent in the start-side (the compartment the animal was initially placed in) and amount of time spent in the tunnel were recorded. Animals were then randomly assigned to drug groups matched for baseline compartment preferences to create an unbiased design. Each group was counterbalanced so that an equal number of animals in each group received drug pairings in either the grate or bar floored compartment. In Phase 2, animals received drug and vehicle injections on alternating days and were restricted to the respective drug- or vehicle-paired compartment for 30 minutes. Following conditioning (Phase 3), CPP was then assessed over three consecutive days. Animals were placed into the initial start-side and allowed free access to both compartments for 15 minutes. The amount of time spent in the start-side and in the tunnel were recorded. The difference in time spent in the drug-paired compartment on the last day of preconditioning and first day of postconditioning was used as the measure of place conditioning.

2.5. Preparation of Drug Solutions

For systemic injections, (±)-7-OH-DPAT HBr (the racemate was used in all studies reported in this thesis) and (+)-amphetamine sulfate were dissolved in 0.9% saline (Fisher Scientific, Nepean, Ontario). Drugs were administered in a volume of 1 mL/kg. For intracerebral injections, 7-OH-DPAT HBr and (+)-amphetamine sulfate were dissolved in 0.9% saline whereas NBQX was dissolved in double distilled water; artificial cerebrospinal fluid (NaCl 147mM, KCl 4.0 mM, $CaCl_2$ 2.3 mM, $MgCl_2$ 1.0 mM, pH 7.2) was used as control solution. All solutions were prepared on the day of injection and doses were expressed as free-base.

2.6. Stereotaxic Surgery

Prior to placing animals into the stereotaxic frame, guide cannulae were positioned at interaural zero and the coordinates were recorded. The stereotaxic coordinates for the NAS shell were anterior +10.1 mm, lateral ±0.2 mm, ventral +3.7mm from interaural zero with the incisor bar set at 2.4 mm below the interaural line (Paxinos and Watson 1986); these coordinated were adjusted to 16 ° lateral relative to the sagittal plane to reduce cereboventricular system damage (Greenshaw 1997). Animals were anesthetized with Halothane and placed in a Kopf stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). A midline incision was made to expose the skull. Four screw holes, two caudal and two rostral of the guide cannulae implantation points, were manually drilled into the skull and stainless steel screws were inserted. A thin layer of dental acrylic was placed around and between the screws to create a base for the implant. Guide cannulae entry points were identified and a Dremel moto-tool (Racine, WI, USA) was used to drill small

holes into the skull until the dura was exposed. The dura was carefully pierced with the tip of a sterile needle and twenty-two gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) were lowered such that their tips were 1 mm above the target injection site. The cannulae were affixed to the initial base with dental acrylic. Stainless steel dummy cannulae (i.e. stylets) were inserted into the guide cannulae to prevent tissue or debris occlusion. The wound was then cleaned and closed with stainless steel wound clips. Animals were placed under a warming lamp until full recovery from the anesthetic. At least one week of recovery was allowed before behavioural testing was initiated.

2.7. Intracerebral Microinjections

Stylets were removed and injection cannulae (26 gauge, Plastics One), connected to polythylene (PE) 10 tubing (Fisher Scientific Co. Pittsburgh, PA, USA) joined to 10 μL microsyringes (Hewlett Packard Co. Palo Alto, CA, USA) mounted on a Bee Hive Controller (Bioanalytic Systems, Inc. West Lafayette, IN, USA), were slowly inserted into the guide cannulae. The injection cannulae protruded 1.0 mm beyond the tips of the guide cannulae. Bilateral infusions (0.5 μL per side) were delivered over 2.5 minutes; the infusion pump was then turned off and injection cannulae were left in place for an additional minute to prevent backflow and allow for drug diffusion. Stylets were then reinserted. To ensure that the correct volume of drug or vehicle solution was injected, a small air bubble was created between the distilled water and drug or vehicle solution in the polyethylene tubing; movement of the bubble during injection verified fluid movement.

2.8. Histology

Following the completion of behavioural testing, animals were deeply anesthetized with sodium pentobarbitol (100 mg/kg, i.p.) and perfused intra-cardially with ice-cold 0.9% saline followed by 10% buffered formalin (Fisher Scientific, Nepean, Ontario). Animals were then decapitated and the guide cannulae were removed at the same angle in which they were implanted in order to reduce tissue damage. The fixed brains were removed and stored in 10% formalin for at least two weeks. To verify cannulae placements, brains were sectioned through the area of the guide cannulae and the most ventral point of the tract was used to denote the injection site. Only animals with correct NAS shell placements were included in the analysis.

2.9. Statistics

Conditioned place preference data for NBQX, 7-OH-DPAT, NBQX+7-OH-DPAT and (+)-amphetamine sulfate were analysed using paired-sample t-tests with statistical significance based on a probability value of $P \leq 0.05$. For locomotor activity, dose-response data were analysed using two-way repeated measures ANOVA with Greenhouse-Geisser correction (drug x time), with a probability value of $P \leq 0.05$ representing statistical significance. The finding of a significant F-ratio was followed by Tukey's Honestly Significant Difference test (HSD). Local time course data were analysed using one-way ANOVA across all treatments at each 5-minute interval. The finding of a significant F-ratio ($P \leq 0.05$) on any 5-minute interval was followed by a *post hoc* comparison of each drug treatment with vehicle using Tukey's Honestly Significant Difference test (HSD) with a significance criterion of $P \leq 0.05$. All statistical analyses

(except Tukey's HSD) were completed using SPSS 10.0 statistical software (SPSS Inc. Chicago, IL, USA) and GraphPad Prism 3.0 (San Diego, CA, USA).

3. EFFECTS OF SYSTEMIC 7-OH-DPAT ON LOCOMOTOR ACTIVITY

3.1. Introduction

The neurotransmitter dopamine mediates a variety of behavioural functions in the central nervous system. Disturbances in dopaminergic neurotransmission have been associated with the etiology of schizophrenia. Early biochemical and pharmacological studies have suggested the existence of two families of dopamine receptors, D_1-like and D_2-like. Molecular biological techniques have identified two dopamine D_1-related subtypes, D_1 and D_5, and three dopamine D_2-like receptor subtypes consisting of D_2, D_3 and D_4 receptors (Emilien et al. 1999). The therapeutic efficacy of typical antipsychotic drugs has been linked to their elevated affinity for dopamine D_2-like receptors; most typical antipsychotics display higher affinity for dopamine D_2 receptors than for dopamine D_3 receptors (Sokoloff et al. 1990; see review by Fink-Jensen 2000). Preferential distribution of dopamine D_3 receptors in limbic regions may be associated with their ability to induce motivated and reward-related behaviours while dopamine D_2 receptors are highly expressed in the caudate nucleus and are implicated in motor control (Levant 1997). The treatment of positive symptoms by typical antipsychotic drugs may be due to their ability to block the stimulation of dopamine D_3 receptors while the alleviation of motor disturbances may be linked to dopamine D_2 receptor manipulation (Schwartz et al. 1993). However, specific dopamine receptor subtypes have not been firmly established in the mediation of motor behaviours. Building on these findings, pharmacological studies have attempted to elucidate the effects of dopamine D_3 receptor agonists on motor output.

Numerous investigators have attempted to understand the role of dopamine D_3 receptors in the mediation of motor behaviours by injecting animals with compounds that have been demonstrated to preferentially bind to dopamine receptors in vitro. One such compound is 7-hydroxy-N,N-di-n-propyl-2-amino-tetralin (7-OH-DPAT), a dopamine D_2/D_3 receptor agonist (Lévesque et al. 1992). A biphasic effect of systemic 7-OH-DPAT on spontaneous locomotor activity has been repeatedly demonstrated in habituated animals; that is, low doses (0.02 to 0.5 mg/kg) of the agonist inhibited or decreased locomotor behaviour while high doses (0.5 to 10 mg/kg) elicited hyperactivity (Daly and Waddington 1993; van den Buuse 1993; Svensson et al. 1994; Ferrari and Giuliani 1995). In turn, variations in the time course of motor behaviours were found between low and high doses of 7-OH-DPAT. Low doses produced a continuous decrease in motor activity while high doses produced an initial decrease in activity, lasting approximately 10 minutes, followed by an increase in locomotion (Khroyan et al. 1995). Starr and Starr (1995) reported that non-habituated mice showed decreased behavioural activity and frozen postures following the administration of 7-OH-DPAT (0.04 to 10.0 mg/kg). In turn, bilateral injections of the agonist, 1.5 and 3 μg/0.5μL, into the NAS suppressed locomotor activity (Ouagazzal and Creese 2000).

Building on existing evidence, the purpose of this experiment was to replicate the dose-dependent effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg) on spontaneous locomotor activity and determine whether the effects were related to the stimulation of dopamine D_3 or D_2 receptor subtypes. A biphasic dose response for 7-OH-DPAT was hypothesized; that is, the higher drug doses, 1.67 and 5.0 mg/kg, would elicit

hyperlocomotion whereas the lower doses, 0.03 and 0.06 mg/kg, would result in hypolocomotion.

3.2. Methods

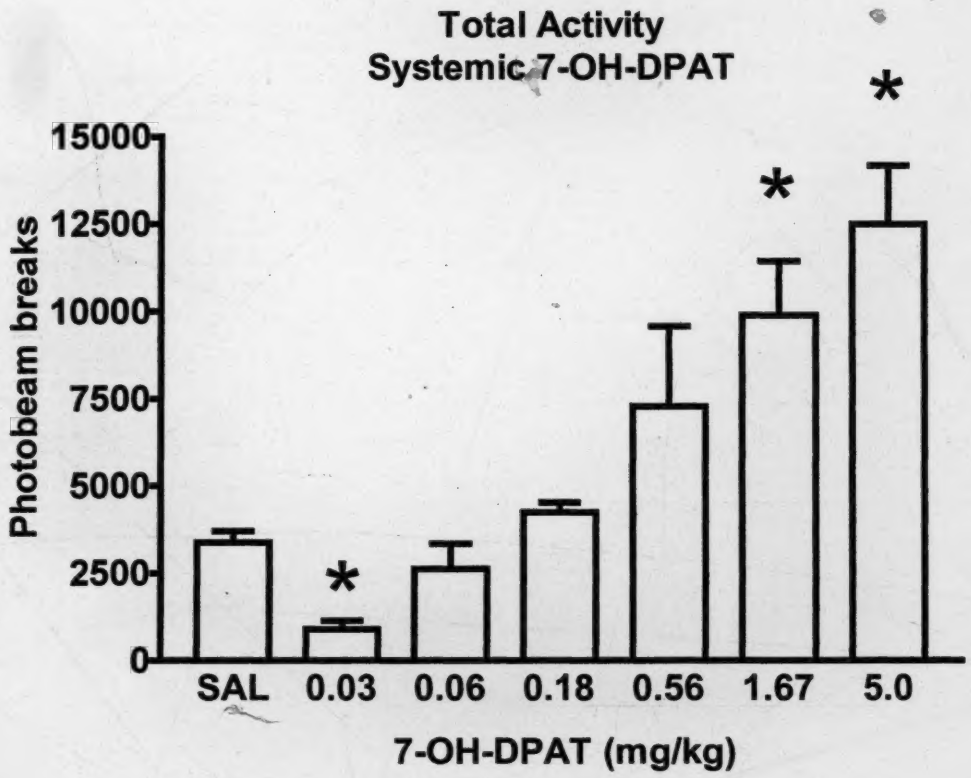
Six male Sprague-Dawley rats were habituated to locomotor activity boxes for 1 hour per day for three consecutive days. A counterbalanced repeated measures design was used in which each animal received seven subcutaneous injections comprised of 0.9% saline, 0.03, 0.06, 0.18, 0.56, 1.67, and 5.0 mg/kg 7-OH-DPAT 15 minutes before being placed in the activity boxes; locomotor activity was monitored for 60 minutes. A three day wash-out period was used between injections.

3.3. Results

Horizontal Activity

Systemic administration of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, and 5.0 mg/kg, s.c.) dose-dependently altered horizontal activity [$F(2.3,11.3)=16.247$, $p<0.05$], Figure 3.1. *Post hoc* tests ($P\leq0.05$) conducted on group mean values (collapsed across time) indicated a significant decrease in total horizontal activity for the lowest dose as well as a significant increase in total activity for the two highest doses compared to vehicle. There was no main effect of time [$F(3.1,15.4)=1.810$, $p>0.05$] nor a significant interaction between 7-OH-DPAT and time [$F(3.5,17.7)=1.607$, $p>0.05$]. Local time course data are shown in Figure 3.2.

Figure 3.1. Effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on total horizontal activity in a 60 minute test session. (n=6). Data are means±S.E.M. *Significant at $P \leq 0.05$, relative to vehicle.



Consecutive Activity

Systemic administration of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, and 5.0 mg/kg, s.c.) dose-dependently altered consecutive activity [$F(2.3,11.3)=9.533$, $p<0.05$], Figure 3.3. *Post hoc* tests ($P \leq 0.05$) conducted on group mean values failed to indicate significant differences between drug and vehicle treatments. There was no main effect of time [$F(2.9,14.7)=3.262$, $p>0.05$] nor significant interaction between 7-OH-DPAT and time [$F(3.9,19.3)=1.593$, $p>0.05$]. Local time course data are shown in Figure 3.4.

Vertical Activity

Two-way repeated measures ANOVA showed no significant main effect of 7-OH-DPAT [$F(1.2,5.9)=2.274$, $p>0.05$], no significant main effect of time [$F(2.5,12.3)=1.432$, $p>0.05$] as well as no significant interaction between 7-OH-DPAT and time [$F(2.6,13.1)=2.806$, $p>0.05$]. Systemic administration of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, and 5.0 mg/kg, s.c.) did not significantly change vertical/rearing behaviour, Figure 3.5. Local time course data are shown in Figure 3.6.

3.4. Discussion

The systemic administration of 7-OH-DPAT dose-dependently altered total and consecutive activity in habituated rats; that is, high doses significantly increased motor activity while low doses resulted in significant hypolocomotion. These findings were consistent with previous work conducted by Daly and Waddington (1993), van den Buuse (1993), Svensson et al. (1994), and Ferrari and Giuliani (1995).

Figure 3.2. Time course effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on total activity in a 60 minute test session. (n=6). Data are means.

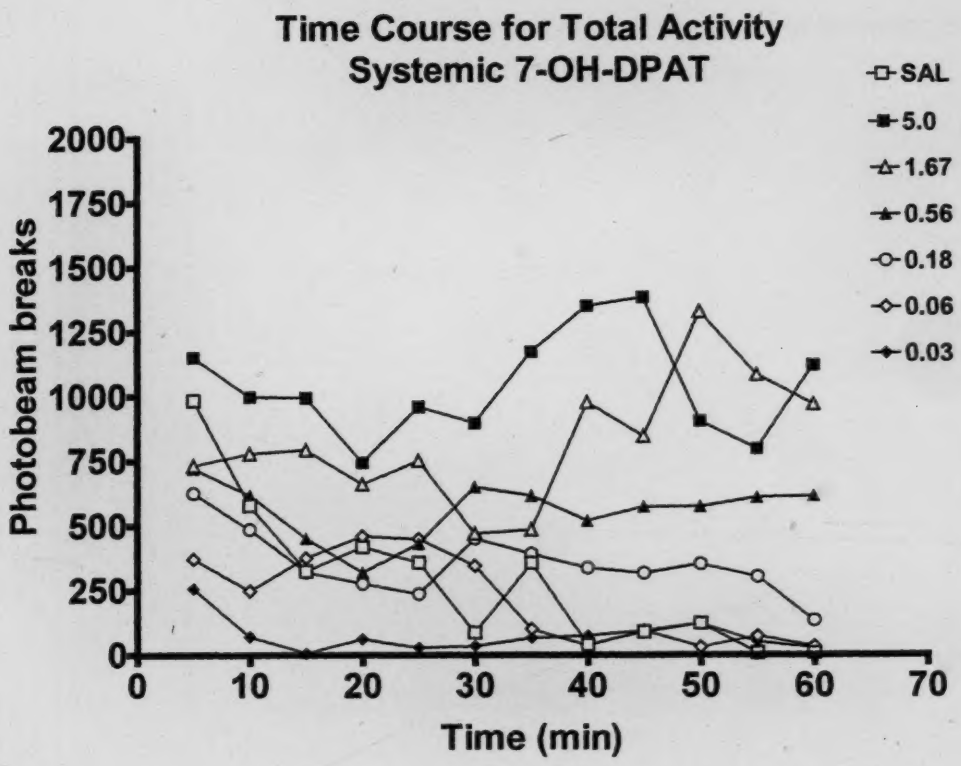


Figure 3.3. Effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on consecutive activity in a 60 minute test session. (n=6). Data are means±S.E.M.

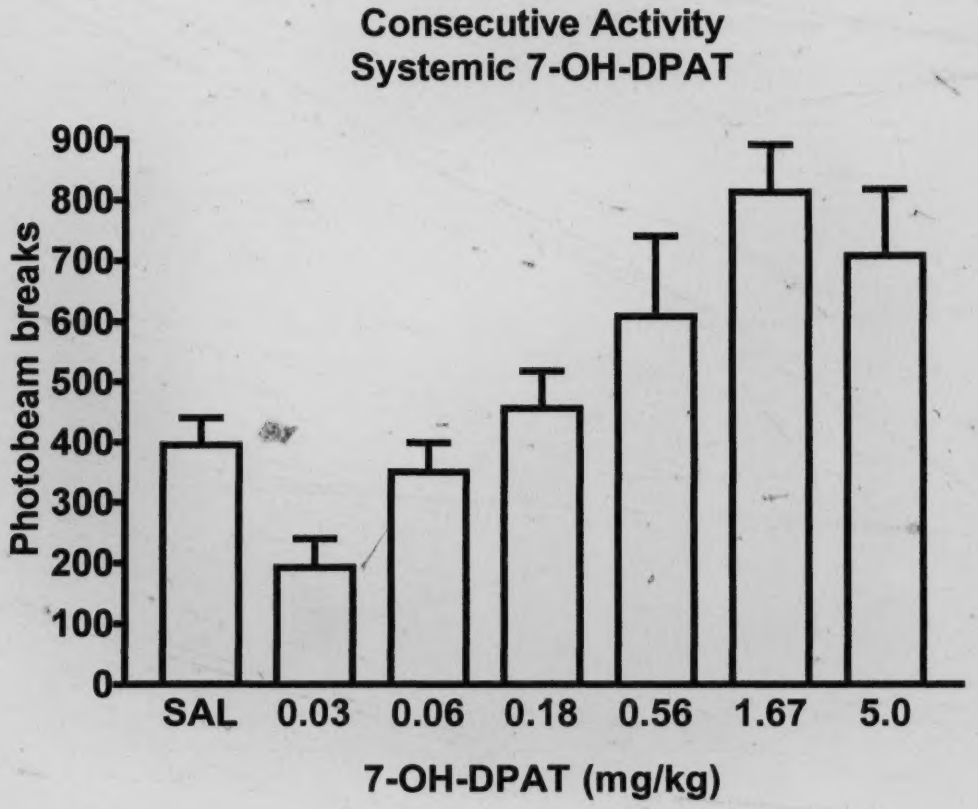


Figure 3.4. Time course effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on consecutive activity in a 60 minute test session. (n=6). Data are means.

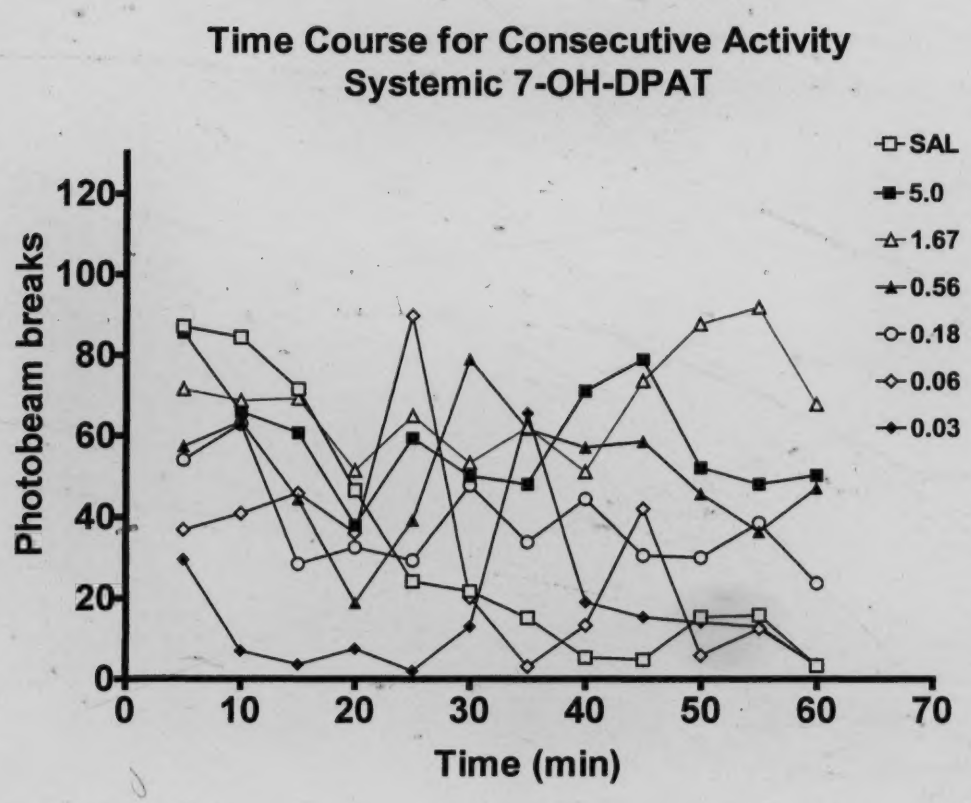


Figure 3.5. Effect of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on vertical activity in a 60 minute test session. (n=6). Data are means±S.E.M.

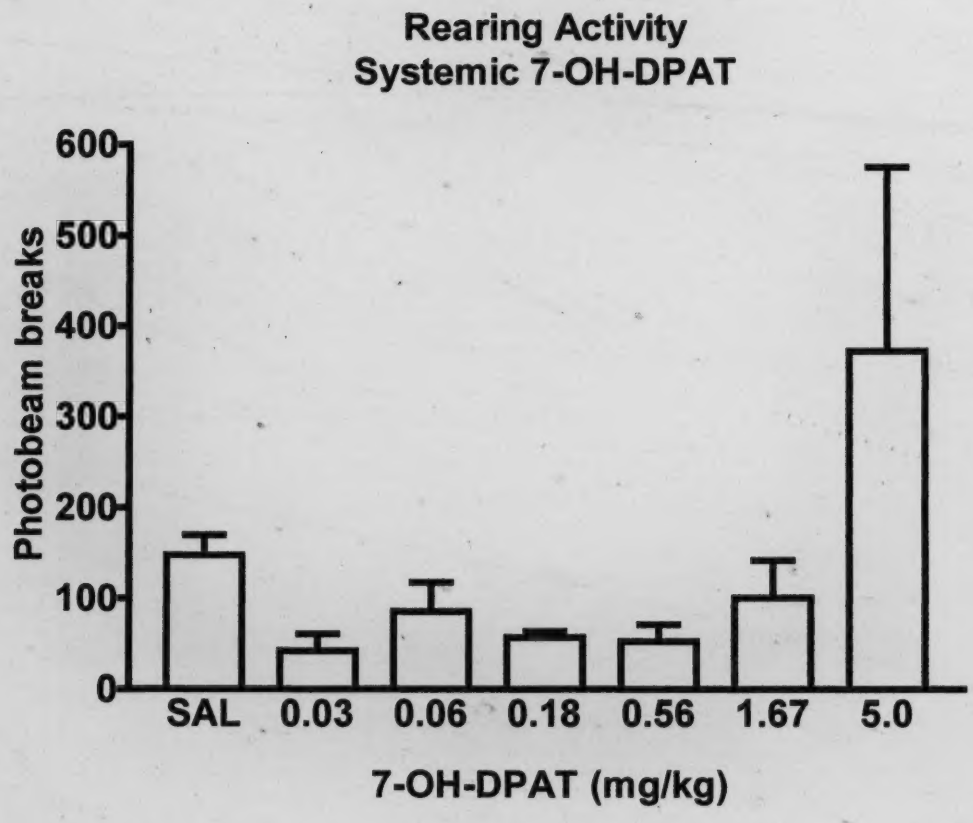
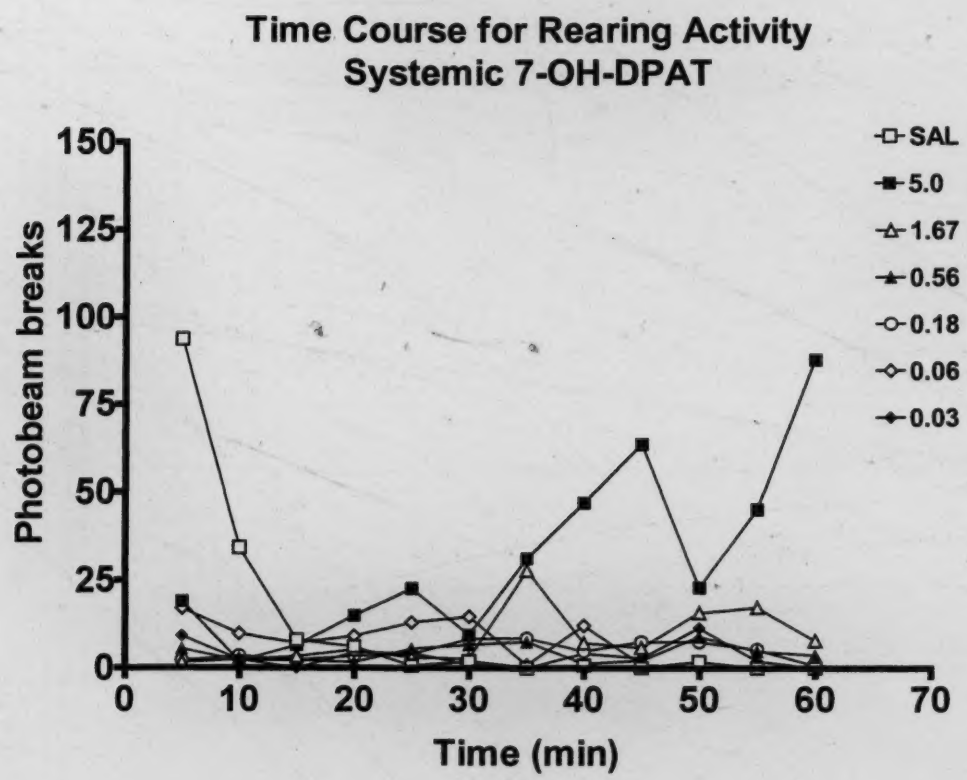


Figure 3.6. Time course effect of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on vertical activity in a 60 minute test session. (n=6). Data are means.



Reduced locomotor activity following systemic administration of low doses of 7-OH-DPAT may be due to a depressant effect caused by a decrease in dopamine release and availability. As dopamine D_3 receptors within mesolimbic regions have been deemed autoreceptors and postsynaptic receptors (Schwartz et al. 1993), decreased locomotor activity may possibly be attributed to the autoreceptor function of dopamine D_3 receptors. A microdialysis study by Damsma et al. (1993) demonstrated an inhibitory action of 7-OH-DPAT that is consistent with stimulation of dopamine D_3 autoreceptors. Sanger and coworkers (1997) found that amisulpiride, an autoreceptor-selective dopamine antagonist, suppressed the discriminative stimulus effects of 7-OH-DPAT. In contrast, Svensson et al. (1994) reported that low doses of R-(+)-7-OH-DPAT produced hypolocomotion but did not alter dopamine release or synthesis, which is associated with autoreceptor activation; these findings indicate the potential involvement of dopamine D_3 postsynaptic receptors. Increases in locomotion by high doses of 7-OH-DPAT may be linked to the activation of dopamine D_2 receptors, as higher doses of the agonist may be necessary for dopamine D_2 receptor binding because of the drug's lower affinity for the dopamine D_2 receptor subtype. Work by Zhang and associates (1997) demonstrated that antisense knockout of dopamine D_3 receptors produced an increase in locomotor activity while antisense knockout of dopamine D_2 receptors decreased locomotor activity. Data from the present experiment suggests that activation of postsynaptic dopamine D_2 receptors may be involved in increases of motor behaviour whereas either dopamine D_3 autoreceptors or postsynaptic dopamine D_3 receptors act to inhibit locomotor activity.

In order to gain a full and accurate understanding of the role that dopamine D_3 receptors may play in the etiology of schizophrenia and their potential therapeutic effects,

further research needs to be conducted across a number of behavioural paradigms with a variety of dopamine D_3 receptor-related compounds. The purpose of the next experiment was to examine the induction of CPP by 7-OH-DPAT as dopamine D_3 receptors, particularly within the NAS, have been suggested to mediate reward-related behaviours.

4. INDUCTION OF CPP BY SYSTEMIC 7-OH-DPAT

4.1. Introduction

Dopamine receptors in the central nervous system have become of significant interest over the past decade as they are believed to play a critical role in numerous psychiatric disorders, such as schizophrenia and Parkinson's disease, as well as mediating drug abuse and reward-related behaviours. Much of the evidence linking dopamine systems to reward has come from pharmacological studies of psychostimulants, dopamine receptor agonists, and dopamine D_1 and D_2 receptor blockade in animals. For instance, drug self-administration studies have reliably induced amphetamine and cocaine (non-selective indirect dopamine receptor agonists) self-administering behaviours in rats (Van Ree et al. 1978; Carroll and Lac 1997). In turn, systemic administration of amphetamine or cocaine produced significant place preference in laboratory animals (Spyraki et al. 1982; Biala and Langwiński 1996). Studies by Self and Stein (1992) and Self et al. (1996) have reported that selective dopamine D_1 agonists, SKF 82958 and SKF 77434, are readily self-administered in rats. Dopamine receptor antagonists, such as pimozide, haloperidol and SCH 23390, have been demonstrated to be effective inhibitors of drug reward (Yokel and Wise 1976; Amit and Smith 1992). Early work by Yokel and Wise (1976) and Roberts and Vickers (1984) demonstrated that systemic administration of pimozide increased self-administration of amphetamine and cocaine in rats, respectively. Building on these findings, Amit and Smith (1992) found that remoxipride, a specific dopamine D_2 receptor antagonist, attenuated amphetamine self-administration. Taken together, these findings suggest that increased dopamine activity within the brain

is a critical factor in the induction of reward-related behaviours. The rewarding properties of drugs acting on dopamine D_1 and D_2 receptors have been extensively characterized using self-administration and conditioned place preference paradigms. The recent cloning of dopamine D_3 receptors has lead to the examination of their role in reward-related behaviours.

Dopamine D_3 receptors have become of particular neuropharmacological interest in the study of reward due to their preferential distribution in limbic regions, specifically in the NAS and islands of Calleja, and the development of dopamine D_3 receptor-preferring compounds, such as 7-OH-DPAT. The dopamine receptor agonist, 7-OH-DPAT, has been demonstrated as having >100-, >1000-, and > 10,000-fold selectivity for dopamine D_3 over D_2, D_4 and D_1 receptors, respectively (Levesque et al. 1992). Studies of in vivo effects of 7-OH-DPAT indicate the involvement of dopamine D_3 receptors in the mediation of reward-related behaviours. The rewarding effects of 7-OH-DPAT have been primarily investigated using self-administration and CPP paradigms. In general, the rewarding effects of the agonist have been shown to vary depending on the dose administered. That is, high doses elicit behaviours similar to those of psychostimulants, such as amphetamine and cocaine, while low doses induce opposite effects. For instance, low doses of 7-OH-DPAT are not self-administered in drug-naïve animals (Caine and Koob 1993; Nader and Mach 1996) and produce conditioned place aversion (CPA) (Khroyan et al. 1995; Chaperon and Thiébot 1996). In contrast, high doses are readily self-administered in laboratory animals (Caine and Koob 1993) and produce CPP (Mallet and Beninger 1994; Kling-Petersen et al. 1995). However, not all evidence supports the dose-dependent effects of 7-OH-DPAT on reward behaviours. De Fonseca and

associates (1995) reported that doses ranging from 0.01 to 5.0 mg/kg did not induce CPP or CPA in rats. The co-administration of 7-OH-DPAT and the indirect dopamine agonists amphetamine and cocaine have also produced inconsistent results. Low doses of the agonist shifted the cocaine self-administration dose response curve to the left, thus indicating reward enhancement (Caine and Koob 1995). In addition, low doses of 7-OH-DPAT attenuated amphetamine and cocaine-induced place preference but potentiated apomorphine-induced CPP (Khroyan et al. 1998; Khroyan et al. 1999). Taken together, these findings suggest that dopamine D_3 receptors may play a role in reward related behaviours, but more conclusive evidence needs to be found.

Differences in methodological procedures may have contributed to the discrepancy in CPP results. For instance, Mallet and Beninger (1994) and Kling-Petersen et al. (1995) reported CPP when 7-OH-DPAT was administered 5 and 10 minutes prior to placement into the CPP apparatus, respectively, while De Fonseca and associates (1995) were unable to elicit place preference or aversion at the same dose when administered immediately before placement into the apparatus. The primary purpose of this study was to further investigate the effects of 7-OH-DPAT on CPP by examining whether the amount of time between injection and placement into the CPP apparatus had a significant effect on CPP induction and whether there was a difference across doses. The second purpose of this study was to try to elucidate the functional differences between dopamine D_2 and D_3 receptors in the context of place conditioning.

4.2. Methods

4.2.1. Experimental design for systemic 7-OH-DPAT, no delay

Forty-two male Sprague-Dawley rats were handled and preconditioned to place preference boxes for 15 minutes per day over three days. They were then randomly assigned to one of seven drug groups matched for baseline compartment preferences to create an unbiased design: 0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg 7-OH-DPAT, s.c., and 5.0 mg/kg (+)-amphetamine sulfate, i.p. Animals received a total of four drug and four saline injections on alternating days and were restricted to the respective drug- or saline-paired compartment for 30 minutes. That is, on days one, three, five and seven of conditioning, animals received an injection of drug and were immediately placed in one of the compartments while on days two, four, six and eight of conditioning animals were injected with saline and immediately placed in the opposite compartment. Each group was counterbalanced so that an equal number of animals received drug pairings in either compartment. Following conditioning, animals were tested for place preference in a drug-free state over three consecutive days.

4.2.2. Experimental design for systemic 7-OH-DPAT, 15 minute delay

Thirty-six male Sprague-Dawley rats were handled and preconditioned to place preference boxes for 15 minutes per day over three days. They were then randomly assigned to one of seven drug groups matched for baseline compartment preferences to create an unbiased design: 0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg 7-OH-DPAT, s.c., and 5.0 mg/kg (+)-amphetamine sulfate, i.p. Animals received four drug and four saline injections on alternating days and were restricted to the respective drug- or saline-paired

compartment for 30 minutes. That is, on days one, three, five and seven of conditioning, animals received an injection of drug 15 minutes before being placed in one of the compartments while on days two, four, six and eight of conditioning, animals were injected with saline 15 minutes prior to being placed in the opposite compartment. Each group was counterbalanced so that an equal number of animals received drug pairings in either compartment. Following conditioning, animals were tested for place preference in a drug-free state over three consecutive days.

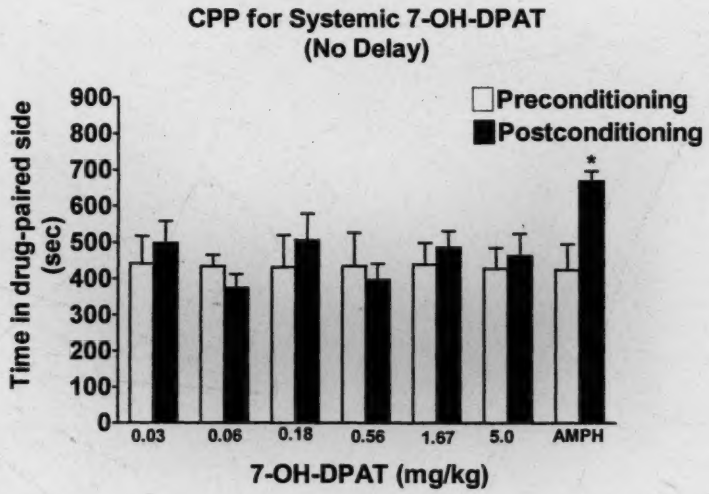
4.3. Results

4.3.1. Failure of systemic 7-OH-DPAT with no delay to induce CPP

Systemic administration of 7-OH-DPAT did not induce CPP or CPA under the no-delay conditioning schedule for any of the doses, Figure 4.1 (A). There was no significant change in the amount of time spent in the conditioned compartment before or after drug treatment for any of the drug doses. However, systemic administration of (+)-amphetamine (5.0 mg/kg) induced a significant place preference [$t(6) = 2.641$, $P<0.05$]. Additional tests performed on time spent in the conditioned compartment revealed significant extinction of (+)-amphetamine place preference for days 2 and 3 of postconditioning, Figure 4.1. (B). That is, amphetamine CPP extinguished by day two of retention testing.

Figure 4.1. Effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., immediately before testing) on the induction of CPP. Each group received four drug and four vehicle injections on alternating days (n=42). Following conditioning, animals were tested for CPP in a drug-free state. (A) CPP with no delay. (B) Extinction of (+)-amphetamine CPP. Data are means±S.E.M. *Significant at $P \leq 0.05$.

A



B

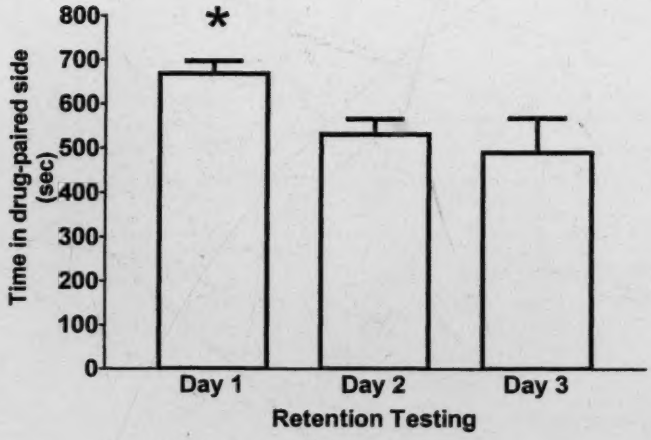
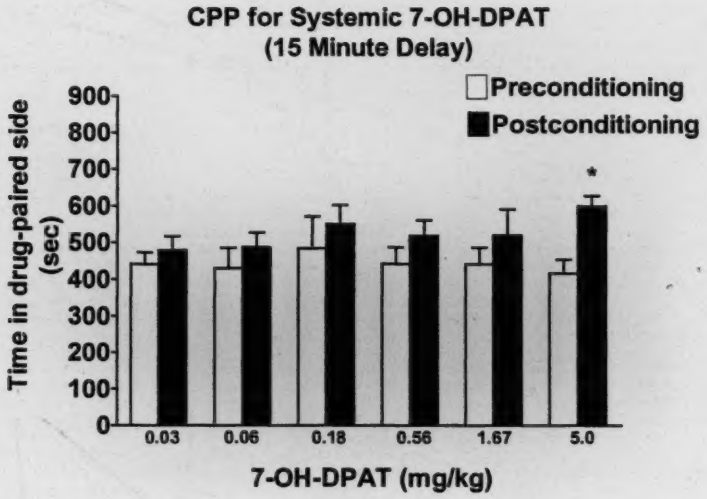
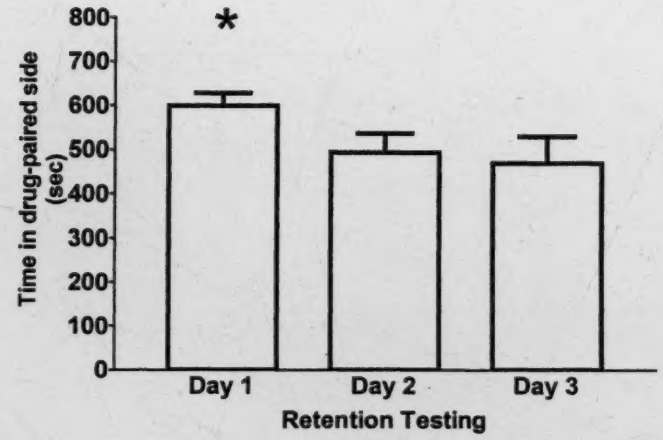


Figure 4.2. Effects of 7-OH-DPAT (0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg/kg, s.c., 15 minutes before testing) on the induction of CPP. Each group received four drug and four vehicle injections on alternating days (n=36). Following conditioning, animals were tested for CPP in a drug-free state. (A) CPP with 15 minute delay. (B) Extinction of 7-OH-DPAT CPP. Data are means±S.E.M. *Significant at $P \leq 0.05$.

A



B



4.3.2. Induction of CPP by systemic 7-OH-DPAT with 15 minute delay

The effects of 7-OH-DPAT on CPP are depicted in Figure 4.2. (A). Treatment with 5.0 mg/kg of 7-OH-DPAT 15 minutes prior to conditioning was sufficient to induce place preference [$t(5) = 3.154$, $P<0.05$]. That is, there was a significant increase in the amount of time spent in the drug-conditioned compartment following drug treatment. Additional tests performed on time spent in the conditioned compartment revealed significant extinction of 7-OH-DPAT place preference on days 2 and 3, Figure 4.2. (B). That is, 7-OH-DPAT CPP extinguished by day two of retention testing.

4.4. Discussion

The dopamine D_2/D_3 receptor agonist 7-OH-DPAT failed to induce CPP for all doses except the highest, 5.0 mg/kg, when administered 15 minutes prior to conditioning. These findings are consistent with data from CPP experiments by Kling-Petersen and associates (1995) as well as locomotor activity data from the previous experiment. Three potential hypotheses may account for these findings. First, the induction of place preference by only the highest dose may be linked to the activation of dopamine D_2 receptors. As the agonist has lower affinity for the dopamine D_2 receptor subtype as compared with dopamine D_3 receptors, higher doses of the agonist may be necessary to activate the receptors. Consistent with this idea, Damsma et al. (1993) and Khroyan et al. (1995) reported that behaviours induced by higher doses of 7-OH-DPAT may involve stimulation of postsynaptic dopamine D_2 receptors whereas lower doses may involve stimulation of postsynaptic dopamine D_3 receptors. The preferential stimulation of dopamine D_3 receptors by 7-OH-DPAT is believed to inhibit behaviours that are

produced by stimulation of dopamine D_2 receptors (Waters et al. 1993; Mattingly et al. 1996); the inhibitory actions of 7-OH-DPAT over a wide range of doses may be due to agonist effects on inhibitory postsynaptic dopamine D_3 receptors (Svensson et al. 1994). Second, behavioural suppression and lack of CPP produced by low doses of 7-OH-DPAT may be due to stimulation of dopamine D_3 autoreceptors that decreases synaptic levels of dopamine (Pugsley et al. 1995). The non-selective dopamine receptor agonist apomorphine has been suggested to induce hypolocomotion by preferential activation of dopamine D_2/D_3 autoreceptors and hyperlocomotion via stimulation of postsynaptic dopamine receptors (Khroyan et al. 1999). Third, endogenous dopamine may be tightly bound to dopamine D_3 receptors in the brain (Schotte et al. 1992); the occupation of dopamine D_3 receptors by endogenous dopamine may reduce their potential activation by the dopamine D_2/D_3 receptor agonist 7-OH-DPAT and may increase the probability that the agonist will act on dopamine D_2 receptors. In summary, the rewarding effects of 7-OH-DPAT in the context of CPP may be produced by activation of dopamine D_2 receptors rather than the dopamine D_3 receptor binding properties of the agonist. The failure of 7-OH-DPAT to elicit reward-related behaviours may be linked to the inhibitory actions of postsynaptic dopamine D_3 receptors.

Interestingly, the highest dose of 7-OH-DPAT only produced place preference when administered 15 minutes prior to conditioning. A possible explanation for the lack of CPP following the no-delay conditioning schedule may be linked to locomotor activity data (refer to Chapter 3). Swerdlow and associates (1989) have proposed that increased locomotor activity facilitates exploration of the CPP apparatus, allowing animals, specifically rats, to become more familiar with distinct spatial stimuli, thereby reducing

environmental novelty; that is, the higher the level of environmental experience the greater the chance of CPP induction. Typically, drugs that stimulate locomotion induce CPP whereas psychomotor depressants induce CPA in rats. In general, locomotor activity studies have reported a significant decrease in locomotion within the first 10 minutes following systemic administration of 5.0 mg/kg of 7-OH-DPAT that is followed by hyperlocomotion (Khroyan et al. 1995). The initial hypolocomotion may occur due to decreased dopamine levels associated with preferential binding of dopamine D_3 receptors while subsequent hyperlocomotion may be the result of dopamine D_2 receptor activation (Khroyan et al. 1995). As the rewarding properties of dopaminergic compounds have been linked to increased dopamine levels within the NAS (Spanagel and Weiss 1999), placing animals into the CPP apparatus 15 minutes post-injection may have increased the likelihood of inducing CPP. In turn, animals immediately placed into conditioning compartments following drug administration may have experienced aversive or non-rewarding states due to decreased dopamine levels that may have counteracted the subsequent rewarding experience. The rewarding effect of a drug may have a temporal profile that is dependent on the time of administration (Ettenberg et al. 1999; Bardo and Bevins 2000).

5. EFFECTS OF NBQX ON 7-OH-DPAT INDUCED CPP

5.1. Introduction

There is considerable evidence that the behavioural effects of reward-related compounds, such as psychostimulants and opiates, are mediated by dopamine-dependent mechanisms; that is, increased dopaminergic neurotransmission within the NAS has been suggested to underlie the rewarding effects (Ikemoto et al. 1997). The NAS is a forebrain structure that receives major dopaminergic input from the VTA and excitatory glutamatergic input from the PFC, hippocampus, thalamus and amygdala (Ikemoto and Panksepp 1999). These projections are believed to converge on the dendritic spines of GABAergic medium spiny neurons that populate the NAS (Sesack and Pickel 1992; Smith and Bolam 1990). This arrangement may provide a basis for interaction between dopamine and glutamate neurotransmitters that may be necessary for behaviours mediated by the NAS, such as locomotor activity and reward.

Numerous *in vitro* and *in vivo* studies have implicated a relationship between glutamate receptors and dopamine receptors in the NAS. In striatal slices, a number of studies have reported excitatory and inhibitory actions of glutamate on dopamine release. Desce and associates (1992) demonstrated that L-glutamate stimulates the release of dopamine whereas Wu et al. (2000) found that activation of ionotropic glutamate receptors in striatal slices inhibited dopamine release. A microdialysis study by Kretschmer (1999) reported that systemic MK-801 increased dopamine release in the NAS and VTA while intra-VTA infusions of NMDA and AMPA elevated dopamine concentrations in both areas. In turn, AP-5 infused into the VTA decreased dopamine release in both the VTA and NAS. A study by Marcus and associates (2001) revealed

that non-competitive NMDA antagonists, PCP and MK-801, evoked dopamine release that was selective to the shell of the NAS; that is, there was elevated dopamine release in the shell but not the core following acute intravenous administration of the non-competitive NMDA antagonists. In turn, observations from behavioural studies may suggest these neurochemical processes.

Several studies have suggested that the mechanisms underlying locomotor activity and reward may involve an interaction between dopaminergic and glutamatergic neurotransmission within the NAS. Willins and colleagues (1992) and Kaddis et al. (1993) reported that intra-NAS injections of DNQX attenuated hyperlocomotion induced by systemic amphetamine and cocaine, respectively. As well, increased locomotor activity produced by amphetamine, cocaine or heroin was attenuated by the NMDA antagonist AP-5 infused into the NAS (Pulvirenti et al. 1991; Kelley and Thorn 1992). Activation of AMPA/kainate receptors as well as dopamine receptors has been investigated in the acquisition and expression of CPP. Layer and co-workers (1993) showed that bilateral injections of DNQX into the NAS suppressed the induction of amphetamine-induced CPP. Kaddis et al. (1995) found that DNQX injected into the NAS blocked cocaine-induced CPP while Cervo and Samanin (1995) reported that DNQX administered intracerebroventricularly had no effect on cocaine-induced CPP while systemic MK-801 blocked cocaine-induced CPP. As cocaine and amphetamine are indirect dopamine agonists, it is unclear whether specific dopaminergic receptor subtypes are differentially affected by glutamatergic antagonism. Therefore, future research needs to investigate the effects of glutamate antagonists on the effects of stimulating or blocking specific dopamine receptor subtypes. AMPA receptors in the NAS may be

involved in the regulation of locomotor activity and reward-related behaviours (Karler et al. 1991; Kaddis et al. 1995). Therefore, the purpose of the following three experiments was to examine the effects of AMPA receptor blockade, by NBQX, on CPP induced by 7-OH-DPAT.

NBQX is a competitive AMPA antagonist that has been implicated in the control of motor activity and reward-related behaviours when systemically or centrally administered to rats. Maj et al. (1995) and Filliat et al. (1998) reported that systemic injections of NBQX, at doses ranging from 5.0 to 30.0 mg/kg, suppressed locomotion and exploratory behaviour in rats. In addition, Maj and associates (1995) demonstrated that the highest dose, 30 mg/kg, blocked hyperlocomotion induced by the NMDA receptor antagonist CGP 37849. Vanover (1998) further reported an attenuation of hyperlocomotion and stereotyped behaviours elicited by amphetamine or MK-801. Mead and Stephens (1999) demonstrated that NBQX had no effect on CPP induced by amphetamine, thus indicating that AMPA receptor antagonism may not contribute to acquisition and expression of conditioned reward. Little research has been conducted on the central effects of NBQX in this regard. Choi et al. (2000) found that intra-NAS shell or core administration of NBQX had no effect on locomotor activity whereas co-administration of the dopamine D_3 agonist 7-OH-DPAT and NBQX into the NAS shell potentiated the locomotor suppressant effects of 7-OH-DPAT. Further research needs to be conducted into the potential synergistic effects of dopaminergic and glutamatergic compounds on reward-related behaviours, such as CPP. Building on findings from the Choi et al. (2000) study, the following experiment investigated the effects of intra-NAS shell infusions of NBQX on 7-OH-DPAT-induced CPP.

5.2. Methods

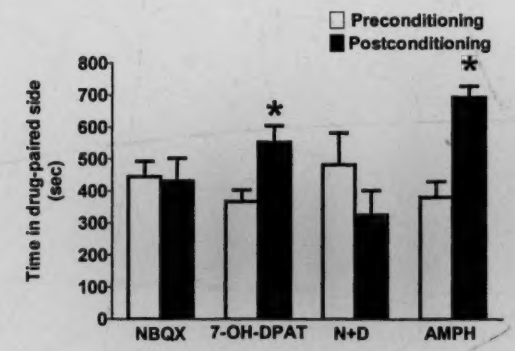
Male Sprague Dawley rats were implanted with bilateral guide cannulae in the NAS shell, as described in Section 2.6. One week post-surgery, animals were handled and preconditioned to the place preference boxes for 15 minutes/day over three consecutive days. They were then randomly assigned to one of four drug groups matched for baseline compartment preferences to create an unbiased design: NBQX (0.5 μg), 7-OH-DPAT (5.0 mg/kg, s.c.), combination of NBQX and 7-OH-DPAT, and (+)-amphetamine sulfate (5.0 mg/kg, i.p.). Each group was counterbalanced so that an equal number of animals received drug pairings in either compartment. All animals received an intra-NAS injection 15 minutes prior to compartment exposure and a systemic injection immediately prior compartment exposure. Animals received four drug and four vehicle injections on alternating days and were restricted to the respective drug- or vehicle-paired compartment for 30 minutes; in this regard, each animal served as its own control. Following conditioning, animals were tested for place preference in a drug-free state over three consecutive days.

5.3. Results

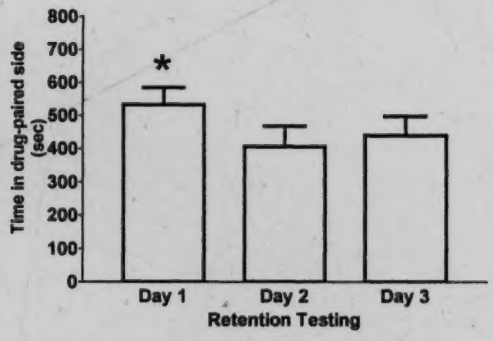
Systemic administration of 7-OH-DPAT (5.0 mg/kg) 15 minutes prior to conditioning induced a significant place preference [$t(4) = 5.471$, $P<0.05$], Figure 5.1 (A). Additional tests performed on time spent in the conditioned compartment revealed significant extinction of 7-OH-DPAT-induced CPP on postconditioning day 2 and 3, Figure 5.1. (B).

Figure 5.1. Effects of intra-NAS shell NBQX (0.5 μg) on the acquisition of systemic 7-OH-DPAT-induced (5.0 mg/kg) CPP. Each group received four drug and four vehicle injections on alternating days (n=21). Following conditioning, animals were tested for CPP in a drug-free state. (A) Drug-free CPP. (B) Extinction of 7-OH-DPAT CPP. (C) Extinction of (+)-amphetamine CPP. Data are means±S.E.M. * Significant at $P \leq 0.05$.

A



B



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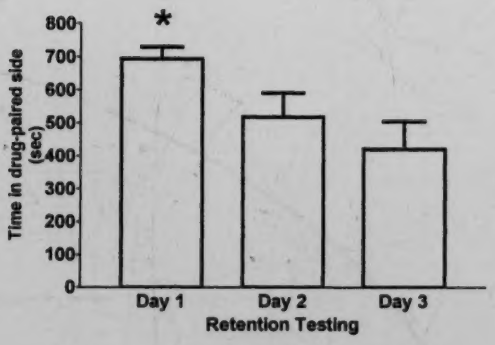


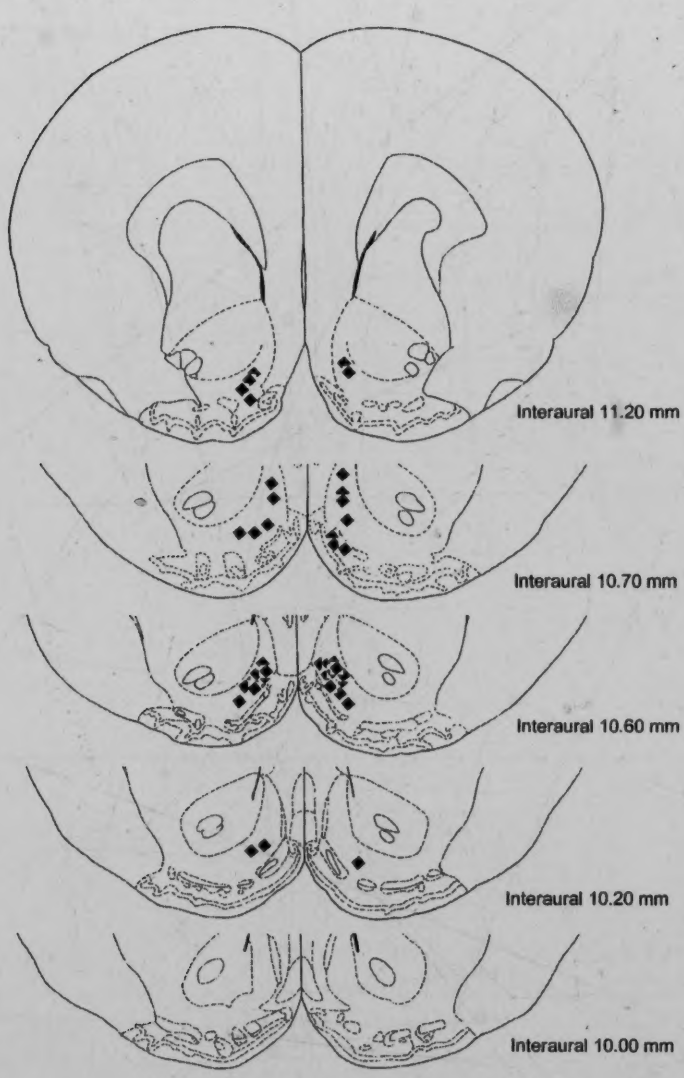
Intra-NAS shell administration of NBQX (0.5 μg) failed to induce significant CPP or CPA when tested in a drug-free state [$t(5) = 0.147$, $P>0.05$], Figure 5.1. (A). That is, there was no significant difference between the amount of time spent in the conditioned compartment before and after drug conditioning. The administration of systemic 7-OH-DPAT followed by intra-NAS NBQX did not induce CPP or CPA [$t(4) = 2.209$, $P>0.05$]. However, systemic administration of (+)-amphetamine induced a significant place preference [$t(5) = 6.733$, $P<0.05$]. Additional tests performed on time spent in the conditioned compartment revealed significant extinction of (+)-amphetamine place preference on day 2 and 3 of retention testing, Figure 5.1. (C).

Histological verifications of microinjection sites in the NAS shell are illustrated in Figure 5.2. Only animals with correct NAS shell placements were included in the analysis.

5.4. Discussion

The failure of NBQX (0.5 μg) to induce place conditioning when injected into the NAS shell is consistent with work conducted on other AMPA/kainate antagonists and agonists (Cervo and Saminin 1995; Gong et al. 1997). For instance, Layer and coworkers (1993) reported that DNQX (1μg/0.5 μL) failed to induce place conditioning as there was minimal change in compartment preference before and after drug conditioning. This phenomenon was also found with NBQX (refer to figure 5.1. (A)); the amount of time spent in the conditioned compartment before and after drug treatment was almost identical, thus indicating that NBQX potentially has little, if any, incentive rewarding properties.

Figure 5.2. Histological verification of microinjection sites in the NAS shell. The numbers represent the distance in the coronal plane from interaural zero according to the modified atlas of Paxinos and Watson (1986).



Gong et al. (1997) demonstrated that the non-NMDA glutamatergic agonist AMPA was unable to elicit place conditioning when bilaterally injected into the ventral pallidum. In addition, brain reward stimulation studies have further supported the view that AMPA receptor-related compounds lack incentive rewarding properties. Choi and Greenshaw (submitted) revealed administration of the AMPA/kainate antagonist CNQX (0.5 μg) into either the NAS shell or core had no significant effect on frequency thresholds of rats responding for VTA electrical stimulation. Building on these findings, recent unpublished data by Clements and Greenshaw indicate that NBQX did not induce significant changes in VTA brain stimulation reward frequency thresholds in rats when injected into the shell of the NAS. Taken together, these findings indicate that AMPA receptor antagonism within the NAS may not be an underlying mechanism of reward.

In agreement with findings from the previous study, systemic 7-OH-DPAT (5.0 mg/kg) produced a significant place preference when administered 15 minutes prior to conditioning. The present study revealed that intra-NAS shell administration of NBQX blocked the acquisition of 7-OH-DPAT-induced CPP. More specifically, coadministration of NBQX and 7-OH-DPAT induced a decrease in preference for the conditioned compartment; however the decrease was not statistically significant to imply CPA [refer to figure 5.1. (A)], which may indicate a synergistic interaction between AMPA/kainate receptors and dopamine D_2/D_3 receptors. As NBQX alone did not induce CPA , the blockade of CPP by 7-OH-DPAT is clearly not an additive effect. Several studies have revealed that AMPA antagonists have the capacity to prevent the expression of CPP induced by psychomotor stimulants that indirectly act on dopamine receptors (Cervo and Samanin 1995; Layer et al., 1993; Tzschentke and Schmidt 1997; Mead and

Stephens 1999). For instance, Cervo and Samanin (1995) and Layer and associates (1993) reported that the AMPA antagonist DNQX blocked the expression of cocaine- or amphetamine-induced CPP in rats, respectively. Similarly, a locomotor activity study by Willins and collaborators (1992) found that hyperactivity induced by systemic amphetamine was attenuated by intra-NAS infusions of DNQX. A more recent study reported that NBQX failed to block CPP induced by amphetamine in mice (Mead and Stephens 1999). The discrepancy in the findings may be due to the use of different species of laboratory animals and differential pharmacological selectivity of the antagonists. However, the ability of intra-NAS shell NBQX to block systemic 7-OH-DPAT-induced CPP suggests that AMPA receptors in the NAS may be involved in reward-related behaviours. Specifically, stimulation of AMPA receptors and activation of dopamine receptors within the NAS may be necessary to facilitate reward-related behaviours.

These data suggest that glutamatergic AMPA/kainate receptors may be involved in the reward circuitry of the NAS in response to the administration of 7-OH-DPAT. The NAS receives major dopaminergic input from the VTA and glutamatergic input from the PFC; these neuronal terminals converge on GABAergic medium spiny neurons. The presence of excitatory input, from the PFC, stimulates dopamine receptors, resulting in prolonged excitatory responses while the absence of excitatory tone produces inhibitory dopaminergic responses and decreased neuronal activity (Kalivas and Nakamura 1999). Blockade of AMPA receptors by NBQX may decrease excitatory tone in the NAS, thereby inhibiting dopamine receptor responses and decreasing dopamine release and neuronal activity. Increased dopaminergic activity within the NAS may be an underlying

mechanism of action by which 7-OH-DPAT elicits CPP; therefore, activation of both dopaminergic and glutamatergic receptors in the NAS may be necessary to produce CPP.

6. FAILURE OF CENTRAL 7-OH-DPAT AND NBQX TO INDUCE CPP

6.1. Introduction

Hyperdopaminergic and hypoglutamatergic functions, particularly within the NAS, have been implicated in the neuropathology of schizophrenia. The NAS has been suggested as an important neural substrate as it receives major dopaminergic afferents from the VTA and excitatory glutamatergic afferents from the PFC (Ikemoto and Panksepp 1999). These innervations are believed to converge on the dendritic spines of GABAergic medium spiny neurons that populate the NAS (Sesack and Pickel 1992; Smith and Bolam 1990). The appositional arrangement of these receptors has been suggested to create a reciprocal relationship between neurotransmitter systems (Svensson et al. 1994; Bunney et al. 1995). In relation to schizophrenia, disruption of glutamatergic input to the NAS, thereby creating a hypoglutamatergic state, may alter the function of dopamine receptors, creating a hyperdopaminergic state, leading to schizophrenic symptoms. To verify this proposition, *in vivo* pharmacological manipulation of the two neurochemical systems within the NAS of laboratory animals needs to be undertaken. The purpose of the final two experiments was to examine the effects of intra-NAS shell AMPA receptor blockade on CPP responses of intra-NAS shell dopamine D_2/D_3 receptor agonist 7-OH-DPAT.

Few studies have investigated the behavioural effects of AMPA/kainate receptor-related compounds within the NAS. Locomotor activity studies conducted by Choi (2000) revealed that administration of the AMPA/kainate receptor antagonists CNQX (0.25 μg and 0.5 μg) and NBQX (0.5 μg) into the either the NAS core or shell did not

alter spontaneous locomotor activity. However, both antagonists further decreased hypolocomotion elicited by intra-NAS administration of 7-OH-DPAT. Brain stimulation reward experiments revealed similar findings. Work by Choi and Greenshaw (submitted) demonstrated that bilateral infusion of either CNQX (0.5 μg) or 7-OH-DPAT (5.0 μg) into the NAS shell did not alter frequency thresholds of rats responding for VTA electrical stimulation. However, a significant increase in threshold frequencies was observed following co-administration of CNQX and 7-OH-DPAT into the NAS shell, indicative of decreased reward. Further research from this laboratory (Clements and Greenshaw, unpublished) has shown elevated frequency thresholds following co-administration of NBQX (0.5 μg) and 7-OH-DPAT (5.0 μg) into the shell of the NAS, suggesting that blockade of AMPA/kainate receptors and stimulation of dopamine D_2/D_3 receptors may act synergistically in the NAS shell to decrease reward. These findings agree with the previous experiment (Chapter 5) in which intra-NAS shell NBQX blocked systemic 7-OH-DPAT-induced CPP. No information is presently available on the effects of centrally administered AMPA/kainate antagonists on CPP induced by the actions of dopamine D_2/D_3 agonists in the NAS shell. The purpose of this experiment was to investigate the effects of intra-NAS shell 7-OH-DPAT, NBQX and NBQX+7-OH-DPAT on CPP induction. Building on the previous results, it was hypothesized that administration of NBQX+7-OH-DPAT into the NAS shell may induce CPA.

6.2. Methods

Male Sprague-Dawley rats were implanted with bilateral guide cannulae in the NAS shell (n= 25), as described in Section 2.6. One week post-surgery, animals were

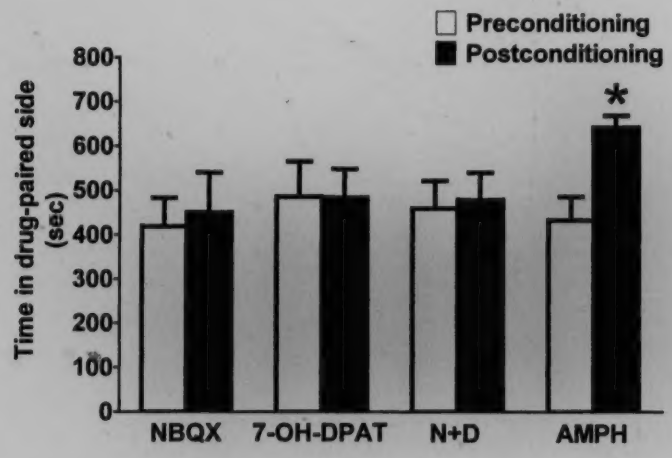
handled and preconditioned to the place preference boxes for 15 minutes/day over three consecutive days. They were then randomly assigned to one of four drug groups matched for baseline compartment preferences to create an unbiased design: NBQX (0.5 μg), 7-OH-DPAT (5.0 μg), combination of NBQX and 7-OH-DPAT, and (+)-amphetamine sulfate (5.0 μg). Drug doses were based on previous work in our laboratory (Choi et al. 2000). Each group was counterbalanced so that an equal number of animals received drug pairings in either compartment. Animals received four drug and four CSF microinjections on alternating days and were restricted to the respective drug- or CSF-paired compartment for 30 minutes; in this regard, each animal served as its own control. Following conditioning, animals were tested for place preference in a drug-free state over three consecutive days.

6.3. Results

The administration of 7-OH-DPAT (5.0 μg) or NBQX (0.5 μg) into the shell of the NAS failed to induce place preference when tested in a drug-free state, [$t(6) = 0.365$, $P>0.05$] and [$t(4) = 1.574$, $P>0.05$], respectively. That is, there was no significant difference between the amount of time spent in the conditioned compartment before and after drug treatment was not significantly different, Figure 6.1. (A). Co-administration of NBQX and 7-OH-DPAT also failed to induce CPP or CPA [$t(6) = 0.752$, $P>0.05$]. However, intra-NAS shell (+)-amphetamine (5.0 μg) induced significant place preference [$t(5) = 3.231$, $P<0.05$]. Additional tests performed on time spent in the conditioned compartment revealed significant extinction of (+)-amphetamine place preference for days 2 and 3 of postconditioning, Figure 6.1. (B). That is, (+)-amphetamine-induced

Figure 6.1. Effects of intra-NAS shell 7-OH-DPAT (5.0 μg), NBQX (0.5 μg), NBQX+7-OH-DPAT or (+)-amphetamine (5.0 μg) on the induction of CPP. Each group received four drug and four vehicle microinjections on alternating days (n=25). Following conditioning, animals were tested for CPP in a drug-free state. (A) Drug-free CPP. (B) Extinction of (+)-amphetamine CPP. Data are means±S.E.M. *Significant at $P \leq 0.05$.

A



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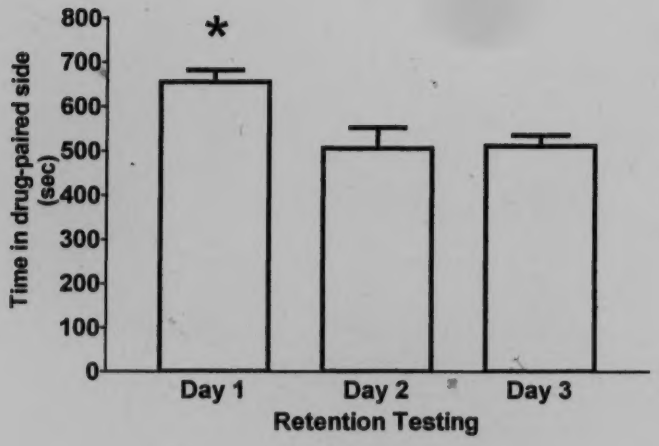


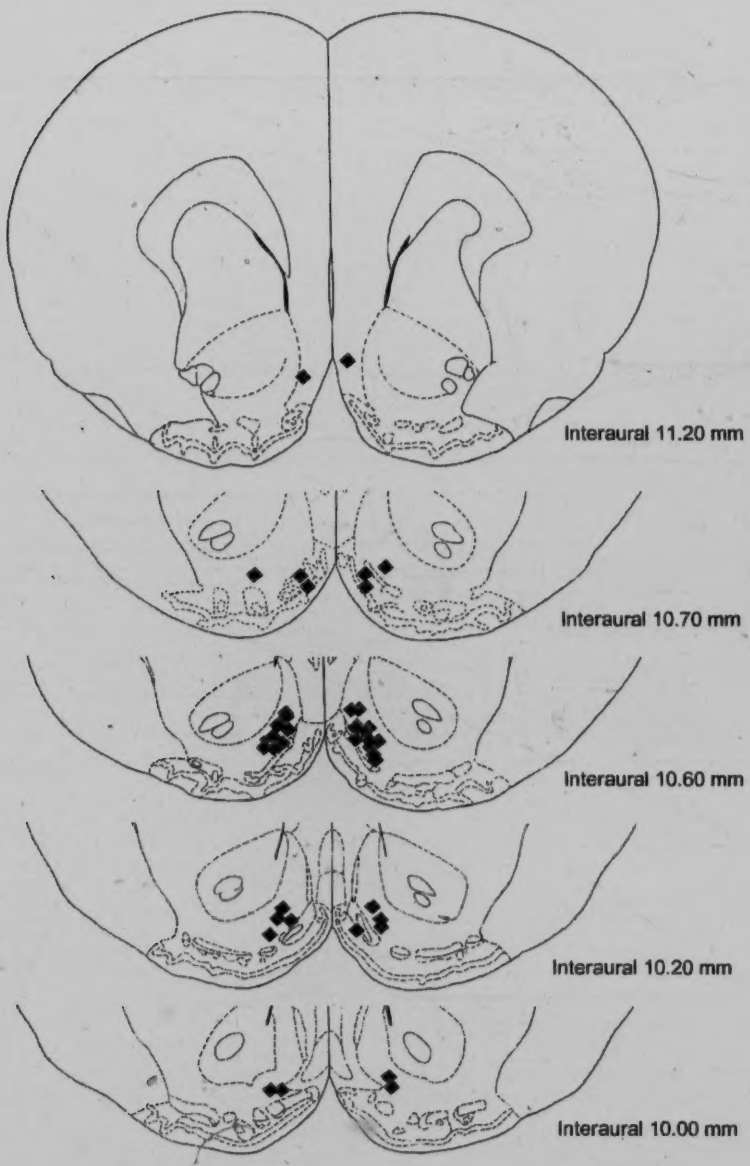
CPP was extinguished by the second day of retention testing.

Histological verification of microinjection sites in the NAS shell is illustrated in Figure 6.2. Only animals with correct NAS shell placements were included in the analysis.

6.4. Discussion

Conditioned place preference is an experimental paradigm for measuring the rewarding nature of drugs. It is based on the idea that an animal may develop a learned association between the rewarding action of the drug and specific environmental cues (in this case, called classical conditioning). The failure of 7-OH-DPAT, NBQX or a combination of these compounds to induce CPP or CPA when injected into the NAS shell may be due to physiological effects of the drugs that may interrupt the formation of a learned association. For example, physiological effects of the drugs could bring about changes in sensory or discriminative abilities that may ordinarily be necessary for the induction of conditioned reward. For instance, numerous studies have indicated that 7-OH-DPAT, when injected into the NAS, decreases locomotor activity and increases levels of sedation (Meyer 1996; Ouagazzal and Creese 2000); the sedative effect of the drug may reduce the ability of the animal to make an association between environmental cues and reinforcing properties of the drug. In turn, the inability to induce CPP may be related to state-dependent learning. In other words, the knowledge acquired while in a drugged state may not be recalled when the animal is not in the same state (Overton, 1978; Tzschentke, 1998). If a drug impairs memory acquisition, CPP or CPA may not be elicited even if the drug is rewarding, as the animal may not learn to make an association

Figure 6.2. Histological verification of microinjection sites in the NAS shell. The numbers represent the distance in the coronal plane from interaural zero according to the modified atlas of Paxinos and Watson (1986).



between the appetitive properties of the drug and environmental stimuli. In order to rule out the confounding effects of state-dependence and drug effects not directly related to reward, one group of animals should be tested in a drug-free state and another under drugged conditions. The purpose of the final experiment was to examine whether the failure to induce place conditioning by intra-NAS shell 7-OH-DPAT, NBQX and 7-OH-DPAT+NBQX was due to state-dependent effects of the drugs.

7. LACK OF STATE-DEPENDENT EFFECTS OF 7-OH-DPAT AND NBQX ON CPP INDUCTION

7.1. Introduction

A primary concern regarding the experimental protocol of CPP is the potential for state-dependent learning or retrieval. State dependency has been referred to as "a response or knowledge that has been learned or acquired while in a given (drugged) state and which can only be obtained or reproduced when the same state is present" (Overton 1978; Tzschentke 1998). Relatively few studies have explored the issue of state-dependency, and those that have often show conflicting evidence. Early research by Reicher and Holman (1977) found that systemic administration of amphetamine produced robust CPP when tested in a drugged or a drug-free state. Similar findings were demonstrated with morphine (Mucha and Iversen 1984), diazepam (Spyraki et al. 1985) and cocaine (Nomikos and Spyraki 1988). In addition, Swerdlow and collaborators (1983) found that cholecystokin-induced (a hormone that when given exogenously had opiate antagonist properties) CPA was produced regardless of whether the animals were tested in a drugged or a drug-free state. Spyraki et al. (1985), however, showed that CPA induced by picrotoxin, a $GABA_A$ receptor antagonist, was state-dependent; that is, CPA was produced only when animals were tested in a drugged state and not under drug-free conditions. A more recent study conducted by Olmstead and Franklin (1997) found that morphine-induced CPP in animals that underwent periaquductal gray or fornix lesions was state-dependent. In contrast, Tzschentke and Schmidt (1997) reported that state-dependency was not responsible for the blockade of morphine-induced CPP by the non-

competitive NMDA receptor antagonist MK-801. In summary, the limited evidence suggests that state-dependent learning may play a role in the development of CPP or CPA and therefore should be further explored. That is, if CPP or CPA is not exhibited under drug-free testing, it is reasonable to test another group of animals in a drugged state to determine if state dependence is a factor.

Despite inconsistent evidence on state-dependency in CPP, it may be necessary to test animals in a drugged state if the drug treatment may potentially produce some degree of memory impairment. As glutamate has been suggested to play a major role in learning and memory, it may be practical and beneficial to evaluate glutamatergic compounds under drugged test conditions. Building on the findings that intra-NAS shell 7-OH-DPAT and NBQX were unable to produce CPP or CPA when tested in a drug-free state, the purpose of this study was to investigate whether the intra-NAS shell administration of 7-OH-DPAT, NBQX or 7-OH-DPAT+NBQX immediately prior to the postconditioning test is necessary to produce CPP; that is, whether state-dependent learning underlies the expression of intra-NAS shell 7-OH-DPAT-induced or NBQX-induced CPP.

7.2. Methods

To test for state-dependency, the same procedure was followed as described in Sections 3.1 and 6.2. with one exception; on the first day of postconditioning, animals received a microinjection of their conditioning drug immediately before being placed into the testing apparatus.

7.3. Results

The administration of 7-OH-DPAT (5.0 μg) or NBQX (0.5 μg) into the shell of the NAS failed to induce CPP or CPA when tested in a drugged state, [$t(6) = 0.019$, $P>0.05$] and [$t(4) = 0.116$, $P>0.05$] respectively. That is, there was no significant difference between the amount of time spent in the conditioned compartment before and after drug treatment, Figure 7.1. (A). Co-administration of NBQX and 7-OH-DPAT also failed to induce a significant CPP or CPA when tested in a drugged state [$t(6) = 1.518$, $P>0.05$]. However, intra-NAS shell (+)-amphetamine (5.0 μg) induced a significant CPP [$t(5) = 2.849$, $P<0.05$]. Additional tests performed on time spent in the conditioned compartment revealed significant extinction of (+)-amphetamine CPP for days 2 and 3 of postconditioning, Figure 7.1. (B). That is, (+)-amphetamine-induced CPP was extinguished by the second day of retention testing.

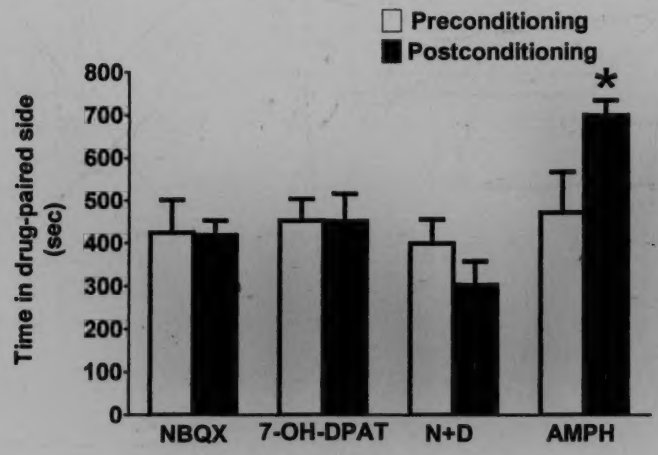
Histological verification of microinjection sites in the NAS shell are illustrated in Figure 7.2. Only animals with correct NAS shell placements were included in the analysis.

7.4. Discussion

The failure of NBQX, 7-OH-DPAT and NBQX+7-OH-DPAT to induce CPP or CPA when tested in the presence or absence of the drug demonstrated that state-dependent learning was not an underlying mechanism of action. The ability of (+)-amphetamine (5.0 μg) to elicit CPP indicated that the CPP procedure used in these studies was capable of establishing CPP; therefore, methodological problems were not responsible for the lack of induction of CPP/CPA by NBQX, 7-OH-DPAT or NBQX+7-OH-DPAT.

Figure 7.1. Assessment of intra-NAS shell 7-OH-DPAT (5.0 μg), NBQX (0.5 μg), NBQX+7-OH-DPAT or (+)-amphetamine (5.0 μg) on the induction of CPP. Each group received four drug and four vehicle microinjections on alternating days (n=25). Following conditioning, animals were tested for CPP in a drugged state. (A) Drug-induced CPP. (B) Extinction of (+)-amphetamine CPP. Means±S.E.M. * Significant at $P \leq 0.05$.

A



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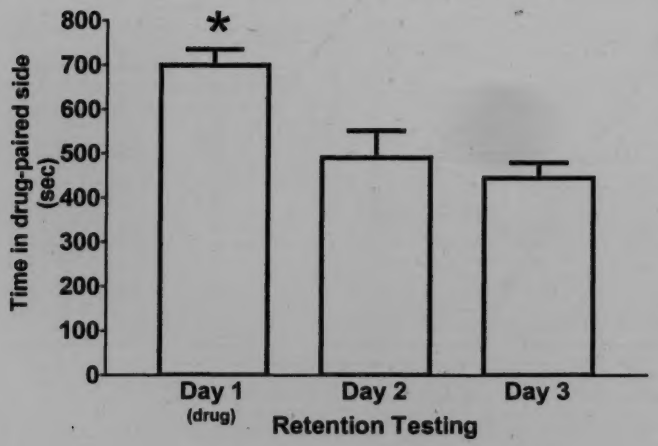
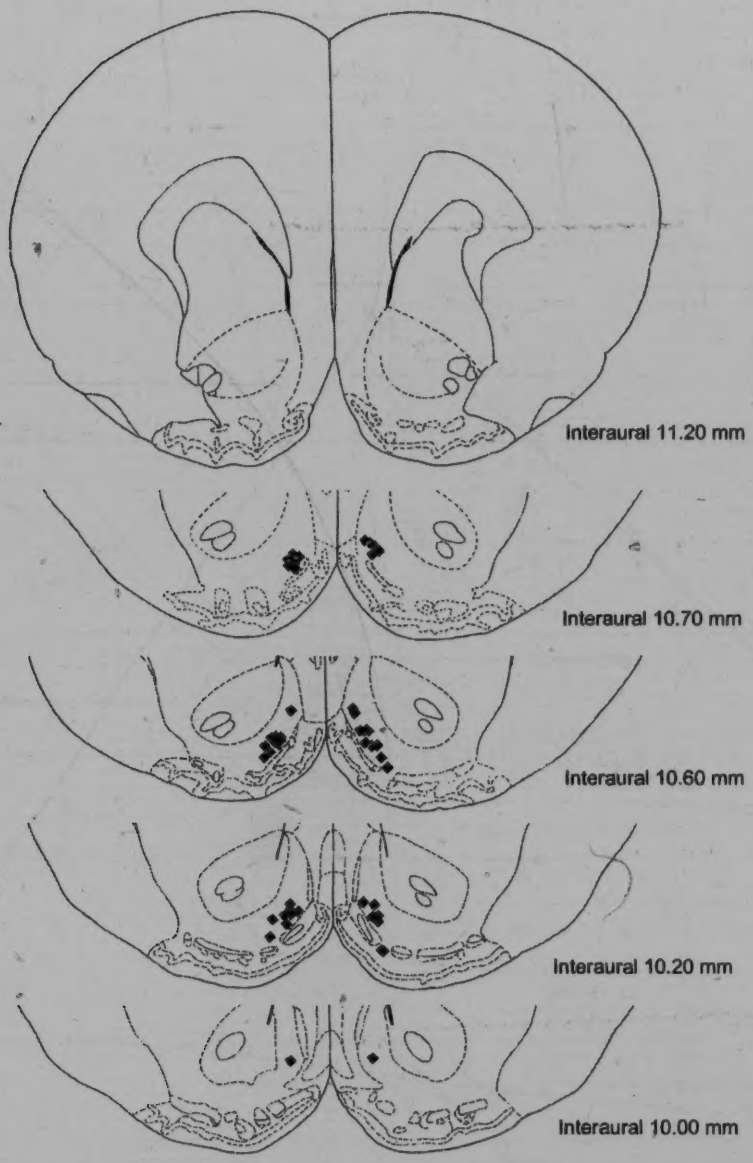


Figure 7.2. Histological verification of microinjection sites in the NAS shell. The numbers represent the distance in the coronal plane from interaural zero according to the modified atlas of Paxinos and Watson (1986).



(+)-Amphetamine was used as an internal control as it has repeatedly and reliably been shown to induce CPP when injected into the NAS. Functional distinction between the core and shell subregions is inconsistent and complex. Deutch and coworkers (1993) have suggested that the shell is responsible for reward-related processes while the core is linked to mediation of motor output whereas Swanson and colleagues (1997) have observed that the NAS shell has greater sensitivity to motor-activating effects of dopaminergic agonists as compared with the core. The majority of research has not examined whether administration of (+)-amphetamine into the NAS core or shell produce differential effects. A recent study by Liao and coworkers (2000) explored this issue and reported that administration of (+)-amphetamine into the NAS core, but not the shell, induced CPP. This is in opposition to findings of the study reported in this thesis in which administration of (+)-amphetamine into the NAS shell induced CPP. Differences in conditioning procedure, drug dose and specific microinjection injection sites may have contributed to the opposing findings of Liao et al. (2000) and the present study. Specifically, Liao and colleagues (2000) conducted 12 days of conditioning, consisting of 6 amphetamine (10.0 μg and 15.0 μg) and 6 vehicle microinjections, as compared with 8 conditioning trials, 4 drug (5.0 μg) and 4 vehicle infusions, in the present work. In turn, injection sites for the present study were located in a more ventral region of the NAS shell as compared to Liao and associates (2000), thereby potentially targeting a different distribution of dopamine receptor subtypes and efferent projections. For instance, dopamine D_3 receptors are most dense along the medial and ventral borders of the NAS shell (Meredith and Totterdell 1999); the medial shell possesses reciprocal connections with the VTA whereas the lateral shell has direct connections with the substantia nigra

pars compacta (Groenewegen et al. 1999). Microinjections of amphetamine into different subregions of the NAS shell may account for the discrepancy between the finding by Liao et al. (2000) and the present study as different subpopulations of dopamine receptors and efferent connections may have been affected.

At the behavioural level, the failure of 7-OH-DPAT to produce CPP when injected into the shell of the NAS is consistent with the finding that it induces hypolocomotion when administered into the NAS (Ouagazzal and Creese, 2000). Increased dopamine release in the NAS has been suggested as an underlying mechanism responsible for hyperlocomotion induced by psychostimulants, opiates and dopamine-related compounds, such as dopamine D_2 agonists. As low doses of 7-OH-DPAT have been shown to decrease locomotion and decrease dopamine release, it is expected that low doses would fail to induce CPP as reward-related behaviours have also been associated with increased dopamine levels. The failure of intra-NAS shell 7-OH-DPAT to produce CPP may suggest that this compound has agonist actions on the postsynaptic dopamine receptors (Svensson et al. 1994); that is, 7-OH-DPAT may increase the inhibitory action of postsynaptic dopamine D_3 receptors, thereby lacking sufficient increases in dopamine concentrations required to induce CPP.

Little is known about the behavioural effects of AMPA and kainate receptor-related compounds. The failure of NBQX (0.5 μg) to induce CPP when injected into the NAS is consistent with the finding that AMPA/kainate receptor antagonists may be inactive in naïve rats (Bubser et al. 1995; Danysz et al. 1994; Li et al. 1997). For instance, Bubser and coworkers (1995) reported that the AMPA antagonist GYKI 52466 did not alter locomotor activity in rats. Open field studies conducted by Danysz et al.

(1994) revealed that numerous AMPA receptor antagonists, such as GYKI 52466 and NBQX, were ineffective in altering locomotor activity.

Several studies have proposed that the behavioural effects of glutamate receptor-related compounds are dependent on interactions with dopamine systems; i.e., the behavioural effects are differentially influenced by the involvement of dopamine D_1 or D_2/D_3 receptors. For instance, Svensson et al. (1992) reported that in monoamine-depleted mice, MK-801 elevated locomotor activity brought about by the actions of dopamine D_1 or indirect dopamine agonists and decreased locomotion when combined with dopamine D_2/D_3 agonists. The failure of intra-NAS shell NBQX+7-OH-DPAT to induce CPP may be due to increased inhibitory action on dopamine D_3 receptors in the NAS brought about by the actions of 7-OH-DPAT. This effect may be compounded by NBQX. That is, administration of NBQX into the NAS shell directly blocked glutamatergic transmission, thus further provoking inhibitory responses of dopamine D_3 receptors, resulting in further decreased neuronal activity; the consequent suppression of dopamine release may be further enhanced by 7-OH-DPAT-stimulated dopamine D_3 autoreceptors or inhibitory postsynaptic receptors which in turn decreases glutamatergic transmission. Thus a reciprocal relationship between dopamine and glutamate within the NAS shell is indicated.

8. General Conclusions and Future Research

Interactions between dopamine and glutamate, particularly in the NAS, have been proposed to underlie the symptoms of schizophrenia as well as mediate reward-related behaviours and motor activity in laboratory animals. Dopamine D_3 receptors and glutamate AMPA receptors are believed to play a central role in reward-related processes. In order to elucidate the role that each plays, an accurate understanding of the basic interplay between the two neurotransmitters and their receptor subtypes must be undertaken. CPP, an indirect measure of drug reward, employs specific antagonists and agonists to identify the involvement of particular neurotransmitters and receptor populations. The purpose of this study was to examine the effects of the dopamine D_2/D_3 receptor agonist 7-OH-DPAT and the glutamate AMPA antagonist NBQX on CPP in order to gain insight into their contributions to reward-related behaviours and, in turn, schizophrenic symptoms.

In the first study, 7-OH-DPAT showed dose-dependent effects on locomotor activity when administered systemically to male rats. That is, high doses (1.67 and 5.0 mg/kg) of 7-OH-DPAT induced hyperlocomotion while low doses (0.03 and 0.06 mg/kg) induced hypolocomotion. The increase in locomotor activity produced by high doses of the agonist may be due to the activation of dopamine D_2 receptors. 7-OH-DPAT has a lower binding affinity for the dopamine D_2 receptor subtype than for dopamine D_3 receptor subtype, so higher does of the drug may be necessary to activate the dopamine D_2 receptor subtype. Hypolocomotion may be linked to dopamine D_3 autoreceptors, which regulate the release and synthesis of dopamine, or inhibitory postsynaptic dopamine D_3 receptors. The inhibitory effect of 7-OH-DPAT may be relevant for the

development of novel antipsychotic drugs because the positive symptoms of schizophrenia have been linked to hyperdopaminergic activity. By activating dopamine D_3 receptors, overall dopamine activity may be decreased and potentially relieve the symptoms. Future research is needed to evaluate a wider variety of dopamine D_2/D_3 receptor compounds and to develop dopaminergic compounds that are selective to the dopamine D_3 receptor subtype.

Inconsistent evidence exists within the literature as to whether 7-OH-DPAT has the capacity to elicit CPP or CPA when administered systemically. Differences in methodological procedures, particularly the amount of time between injection and placement into the CPP apparatus as well as drug dose administered, appear to be the underlying factors that contribute to these inconsistencies. The purpose of the second study was to clarify the ability of systemic 7-OH-DPAT to induce CPP by examining whether these methodological differences had an effect on CPP/CPA induction. 7-OH-DPAT failed to induce CPP or CPA for all doses except the highest, 5.0 mg/kg, when administered 15 minutes prior to conditioning. This indicates that the temporal profile of the drug may influence its ability to elicit CPP. As with the previous locomotor activity study, the induction of CPP by the highest dose may be linked to the activation of dopamine D_2 receptors while the lack of place conditioning at lower doses may be due to the inhibitory action of postsynaptic dopamine D_3 receptors. These findings indicate that the dopamine D_2 receptor may be the receptor subtype responsible for reward-related behaviours, such as drug abuse.

In contrast, intra-NAS shell administration of 7-OH-DPAT (5.0 μg) failed to induce place conditioning. This effect may be related to the dose of 7-OH-DPAT used,

as the development of CPP or CPA is dependent upon the sufficient drug-induced changes in the internal affective state of the animals. If the dose of a compound is too high or too low to elicit sufficient internal affective changes, then CPP/CPA may not be induced; that is, the induction of CPP by many drugs is often dose-sensitive. A wider dose-response analysis for 7-OH-DPAT on CPP would help to clarify the rewarding nature of the compound.

The induction of CPP by systemically administered 7-OH-DPAT suggests that the net rewarding effect of the drug may potentially be due to actions in multiple brain areas. The VTA has been found to possess an abundance of dopamine D_2 and dopamine D_3 receptor subtypes (Jackson and Westlind-Danielsson 1994) and has been implicated in the modulation of reward and motor behaviour (Kalivas and Nakamura 1999). Activation of dopamine receptors in both the VTA and NAS, and potentially other brain structures, may be necessary to induce reward-related behaviours. In turn, systemically administered drugs may affect different subpopulations or subtypes of receptors than centrally administered compounds. As 7-OH-DPAT is an agonist at both dopamine D_2 and D_3 receptors, the discrepancy in CPP induction may be due to activation of different receptor subtypes. Locomotor activity studies have demonstrated that activation of dopamine D_2 receptors increases locomotor activity whereas stimulation of dopamine D_3 receptors inhibits locomotor activity (Levant 1997; Shafer and Levant 1998). For example, Svensson and associates (1994) reported that dopamine D_2/D_3 agonists that possessed a higher affinity for the dopamine D_3 receptor subtype, such as pramipexole, were more efficacious in decreasing locomotor activity than dopamine D_2 preferring agonists, such as 3-(3-Hydroxyphenyl)-N-propylpiperidine. Consistently, Sautel and

coworkers (1995) observed that nafadotride, a dopamine D_3 receptor-preferring antagonist, produced hyperactivity at low doses and inhibited locomotor activity at high doses. However, findings from the latter study have not yet been replicated.

Numerous studies have reported a synergistic interaction between dopamine and glutamate within the NAS; that is, dopamine and glutamate have been proposed to have a reciprocal relationship. In particular, blockade of glutamate receptors results in increased dopamine activity. A model by Harvey and Lacey (1997) proposed that stimulation of dopamine D_1 receptors indirectly inhibits glutamatergic neurotransmission within the NAS via NMDA receptor-mediated adenosine release. As a result, no additional effects of AMPA receptor blockade can be generated following dopamine D_1 receptor stimulation. It should be noted that this model does not provide a mechanism of action for dopamine D_2 and dopamine D_3 receptor agonist interaction with AMPA receptor antagonism and that as that it is inconsistent with other behavioural findings. For instance, White (2001) reported that dopamine D_1 receptor stimulation enhances AMPA receptor activity via postsynaptic effects, mainly AMPA receptor phosphorylation. The question still remains as to whether dopamine D_2 and dopamine D_3 receptor activation may contribute to AMPA receptor blockade or vice versa.

The final three experiments focused on examining the relationship between AMPA receptor blockade, by NBQX, and dopamine D_2/D_3 receptor activation by 7-OH-DPAT in the context of reward. NBQX failed to induce CPP or CPA when bilaterally infused into the shell of the NAS; this finding is consistent with CPP studies using other AMPA receptor antagonists as well as with data from brain stimulation reward studies (Choi 2000). Thus, AMPA receptor antagonists may not possess sufficient incentive

rewarding qualities necessary to induce CPP. However, intra-NAS shell administration of NBQX blocked the acquisition of systemic 7-OH-DPAT-induced CPP. These findings suggest a functional relationship between glutamatergic AMPA receptor blockade and stimulation of dopamine D_2/D_3 receptors in the NAS shell in the context of reward, as described below.

The blockade of 7-OH-DPAT-induced CPP following co-administration with NBQX suggests a synergistic relationship between AMPA glutamate receptors and dopamine D_2/D_3 receptors within the shell of the NAS. Blockade of glutamate AMPA receptors within the NAS shell by NBQX may cause a decrease in glutamatergic transmission; this decrease in excitatory input into the NAS shell may provoke inhibitory responses in dopaminergic neurons, resulting in decreased neuronal activity. In addition, the inhibitory actions of dopamine receptors are further enhanced by 7-OH-DPAT, which in turn further decreases glutamatergic transmission. That is, the decrease in preference for the conditioned compartment following the co-administration of NBQX and 7-OH-DPAT may reflect a potentiation of dopamine D_2/D_3 receptor effects by AMPA receptor blockade by NBQX. This finding is consistent with brain stimulation reward studies from our laboratory (Choi and Greenshaw submitted; Clements and Greenshaw unpublished) that demonstrate the co-administration of 7-OH-DPAT and either CNQX or NBQX act synergistically to significantly increase frequency thresholds of rats responding for electrical stimulation of the VTA, suggesting a failure to facilitate reinforcing behaviour. The behavioural inhibition elicited by stimulation of dopamine D_2/D_3 receptors and the synergistic interaction between glutamate AMPA receptors and dopamine D_2/D_3 receptors within the NAS may have significant implications for

understanding the neuropathology of schizophrenia and drug abuse as well as providing a new focus for the development of antipsychotic drugs.

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